**Heading/Page Format:**

1. Appropriate demographics including name of the institution, department name, section

name and address. (example: Hospital XYZ, Core Laboratory, Chemistry / Hematology section etc.

2. Name of individual/author who wrote the procedure

3. Name of the procedure that the new procedure replaces, when applicable.

4. Month and year the procedure is adopted.

5. Page number and total number of pages in the document (example: Page 1 of 10)

Bilirubin in urine determination by **DIAZO-CHEK** (Biorex Labs) method.

**Principle:**

The test is based on the diazo coupling reaction between the adsorbed bilirubin and a stabilized diazonium salt of Nitrobenzene. The reaction results in the development of varying grades of purple to bluish-purple color which is easily visible by the naked eye.

**Clinical Significance**:

 The presence of bilirubin in urine implies liver disorder and can be an early feature of obstructive hepatobiliary disease. The DIAZO-CHEK test is indicated to detect small amounts (~0.1 mg/dl or greater) of bilirubin in urine which may appear in the earliest stages of hepatic disease.

# Specimen:

# Freshly passed or refrigerated urine. Bilirubin is a labile molecule and breaks down rapidly once excreted in urine. Since it is particularly sensitive to heat and light, the specimen should be refrigerated for no longer than 4-6 hours if not tested immediately. The commonly used urinary preservatives do not prevent its decomposition.

**Reagents and supplies** :

1. Test Pad squares
2. Reagent discs
3. Forceps
4. Reusable micropipettes (to dispense water only)

**Quality Control**: Perform quality control according to your institutional guidelines. At least a weekly QC is strongly recommended.

**Procedure:**

1. Place a Test Pad Square (supplied with the kit) on a piece of gauze or paper towel.
2. Gradually place 4 drops of urine onto the center of the square using a disposable transfer pipette.
3. With a forceps remove reagent disc from the jar and place it in the center of the moistened test pad square. Recap the discs jar promptly and securely.
4. With a plastic micropipette (provided) gradually (wait a couple of seconds between the drops) add two drops of distilled water (~ 20-25 l each) onto the disc (micropipettes provided in the kit).
5. Read the results at 60 seconds; use a timer.

**Reporting Results:**

If the pathological level of bilirubin is present in the urine specimen a purple color develops around the disc within 60 seconds. The rapidity of the purple color development and its intensity is proportional to the amount of bilirubin present in the specimen. The normal urine contains ~0.02 mg/dl of bilirubin which gives a negative result with DIAZO-CHEK method. All colors other than purple or bluish purple should be ignored. A slight pink or red color is also a negative test. A light brown or tan halo is a negative test as well.

Report results as positive or negative

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#  Two representative positive test results for urine bilirubin.

**Limitations:** Chloropromazine and Etodolac (NSAID) metabolites in urine may cause unusual or false positive results. Metabolites of Phenazopyridine produce bright orange color urine and maymask the color from small amounts of bilirubin present in the urine.

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