Urobilinogen Test GENLISA™ Assay

(colorimetric method) Cat No: KBCA2381 Pack: 100T

1. Determination principle:

Urobilinogen reacts with p-dimethylaminobenzaldehyde in an acidic solution to form a red compound.

2. Reagent composition: (50 tubes/48 samples)

Reagent 1: 100 g/L barium chloride solution 100 mL x 1 bottle (can be used with urine bilirubin reagent), stored at 4°C.

Reagent 2: Hydrogen peroxide, 10 mL x 1 bottle, store at 4°C.

3. Operation steps:

- 1. Add 1.5 mL of reagent 1 (barium chloride) to 3 mL of urine, mix well, and centrifuge at 3000 rpm for 5 minutes. (If you have purchased the urine bilirubin kit of our institute, the supernatant after centrifugation can be used to measure urobilinogen, and the precipitate can be used to measure urine bilirubin.)
- 2. Take out the supernatant, add 0.3 mL of reagent 2 (urobilinogen reagent), let it stand at room temperature for 5 to 10 minutes, and observe from the tube mouth to the bottom of the tube (vertically) (a white porcelain plate or white paper can be placed at the bottom of the tube).

4. Result analysis:

- 1. Strong positive (3+), immediately showing deep red;
- 2. Positive (2+), cherry red;
- Weakly positive (+), microscopically red;
- 4. Negative (-), no red color and no red color after heating.

Normal urine should be weakly positive, or positive (+) after slightly warming. If it is positive (++), the urine should be diluted 20 to 160 times with water and then measured according to the above method.

5. Notes:

- 1. Urine must be fresh, avoid sunlight, and be tested in time, otherwise urobilinogen will be oxidized into urobilin.
- The color development speed and depth of the test are greatly affected by temperature. Generally, the test should be carried out at around 20°C. If the room temperature is too low, it must be heated.
- 3. Adding the reagent to alkaline urine will produce yellow turbidity. At this time, dilute hydrochloric acid should be added to make it close to neutral.
- 4. If a large amount of broad-spectrum antibiotics are used, the reproduction of intestinal bacteria can be inhibited, so that bilirubin cannot be reduced to urobilinogen, and the urobilinogen in the urine is reduced or becomes negative.
- 5. Various drugs in urine can react with the reagent. For example, formaldehyde or hexamethylenetetramine can inhibit this reaction. If sulfonamide drugs are contained in urine, the urine will turn yellow after adding the reagent, and may even produce yellow-red precipitation. Thiophene drugs such as chlorpromazine can turn purple or red. For the identification methods of other urobilinogens, please refer to the determination of bilirubinogen.
- 6. In normal people, the amount of urobilinogen excreted increases rapidly in the afternoon, reaching its peak 2 to 4 hours in the afternoon.



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