Chapter 6

CHEMICAL EXAMINATION OF URINE

Reagent Strips

Most common method for chemical testing

Plastic strip with reagent-impregnated pads dipped in urine, resulting in a visual color change

Qualitative results based on color change

Specificity and sensitivity of each test varies between manufacturers

Some interfering substances such as ascorbic acid need to be detected or eliminated

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Quality, Care, and Storage

Strips must be protected from moisture, heat, chemicals, and light with tight-fitting lids and desiccant in container

Store in original container below 30°C

Follow manufacturer's directions and timing for reading colors precisely

New containers or lot numbers of reagent strips must be checked with quality control materials at a frequency defined by the laboratory in conformity with applicable regulations

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Tablet and Liquid Chemical Tests

Must be handled and stored according to manufacturer's product inserts

Products are susceptible to light, heat, and moisture and should be checked for deterioration before each use

Appropriate quality control must be employed when using tests

When new liquid reagents are prepared, they should be tested in parallel with the current "in-use" reagents to ensure equivalent performance

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BOX 6.1 Appropriate Manual Reagent Strip Testing Technique

Room conditions: Good lighting, preferably fluorescent; avoid direct sunlight

Urine specimen: At room temperature

Technique:

1. Using *uncentrifuged* urine, mix specimen well.
2. Dip reagent strip *briefly* into urine to wet all reaction pads and start timing device.
3. Remove excess urine by drawing edge of strip against rim of container or by blotting strip edge on absorbent paper.
4. At the appropriate times, read results of each reaction pad using the color chart on the container.
5. Discard strip into biohazard waste.

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Reasons to Use Tablet/Liquid Tests

Confirm results obtained by reagent strip testing

Alternative method for highly pigmented urine that may make reagent strip result interpretation more difficult

Some tests are more sensitive than strip testing (Ictotest tablets for bilirubin)

Test specificity differs from strip method (sulfosalicylic acid [SSA] test for protein)

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Specific Gravity (SG)

An indirect chemical method of measuring SG

Only measures ionic solutes (sodium [Na], potassium [K], chloride [Cl], ammonium [NH_4])

Principle:

- Reagent strip pad impregnated with a polyelectrolyte and a pH indicator at an alkaline pH
- When strip immersed in urine, protons are released from polyelectrolyte in proportion to ionic concentration
- Released protons change pH of test pad resulting in a color change of pad

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pH

Normal pH varies from 4.5 to 8.0; usually slightly acidic but more alkaline after meals

pH can affect stability of formed elements

Should be performed on fresh urine

Principle:

- Based on a double indicator system
- Uses bromothymol blue and methyl red
- Produces color change from orange (pH 5.0) to green (pH 7.0) to blue (pH 9.0)

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Blood

Terminology:

- Hematuria
  - Red blood cells (RBCs) in urine
- Hemoglobinuria
  - Free hemoglobin in urine

Detects hemoglobin heme moiety, so reagent strip also detects myoglobin

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Blood (Cont.)

Based on heme's pseudoperoxidase activity

- Pad has chromogen and peroxide
- Pseudoperoxidase activity of heme reduces peroxide, and chromogen is oxidized, causing color change from yellow to green
- Ascorbic acid is known to interfere with reaction and can cause a false-negative reaction, suggested by positive microscopic findings but a negative reagent strip result

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Leukocyte Esterase

Normally, few white blood cells (WBCs) seen in urine; equivalent to 0 to 8 per high-power field or approximately 10 WBCs per microliter

Greater than 20/µL is an indication of pathologic process

WBCs susceptible to lysis, so may not be seen but will release enzyme causing a positive reagent strip result

Principle based on action of leukocyte esterase to cleave an ester in pad

- Cleavage forms an aromatic compound, which couples with a diazonium salt in test pad
- End result is azo dye and color change from beige to violet
- Able to detect 10 to 25 WBCs/µL

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Nitrite

Nitrate-reducing bacteria in urine can form nitrite

Requirements:

- Bacteria present must be nitrate reducers
- Adequate time in bladder to be reduced (4 hours)
- Adequate dietary nitrate intake

Principle based on diazotization reaction of nitrite with an aromatic amine in pad

- Forms a diazonium salt, which couples with an aromatic compound in pad to produce azo dye
- Color change white to pink

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Protein

Normally very small amounts of small-molecular-weight proteins in urine; no large proteins

Urine protein often first sign of kidney disease

Strip test most sensitive to albumin

Principle based on the protein error of indicators:

- When buffers in pad hold pH constant, certain indicator dyes release H ions due to proteins present
- Hydrogen (H) ions combine with protein, causing a color change
- Intensity of the color proportional to protein amount

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Microalbumin (Low Levels of Albumin)

Routine test strips unable to detect albumin in urine that is less than 1 to 2 mg/dL

Sensitive albumin tests detect low-level albuminuria

Variety of test methodologies:

- Monoclonal antibodies
- Chemical reactions with dye binding

Most often used in patients with diabetes to screen for signs of early kidney damage

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Glucose

Not seen in normal urine

Will appear if plasma level in urine is over threshold level of 160 to 180 mg/dL

Principle based on a double sequential enzyme reaction that is specific for glucose:

- Glucose oxidase in pad oxidizes glucose to form hydrogen peroxide and gluconic acid
- Peroxidase in pad catalyzes formed hydrogen peroxide to oxidize chromogen in pad resulting in a color change

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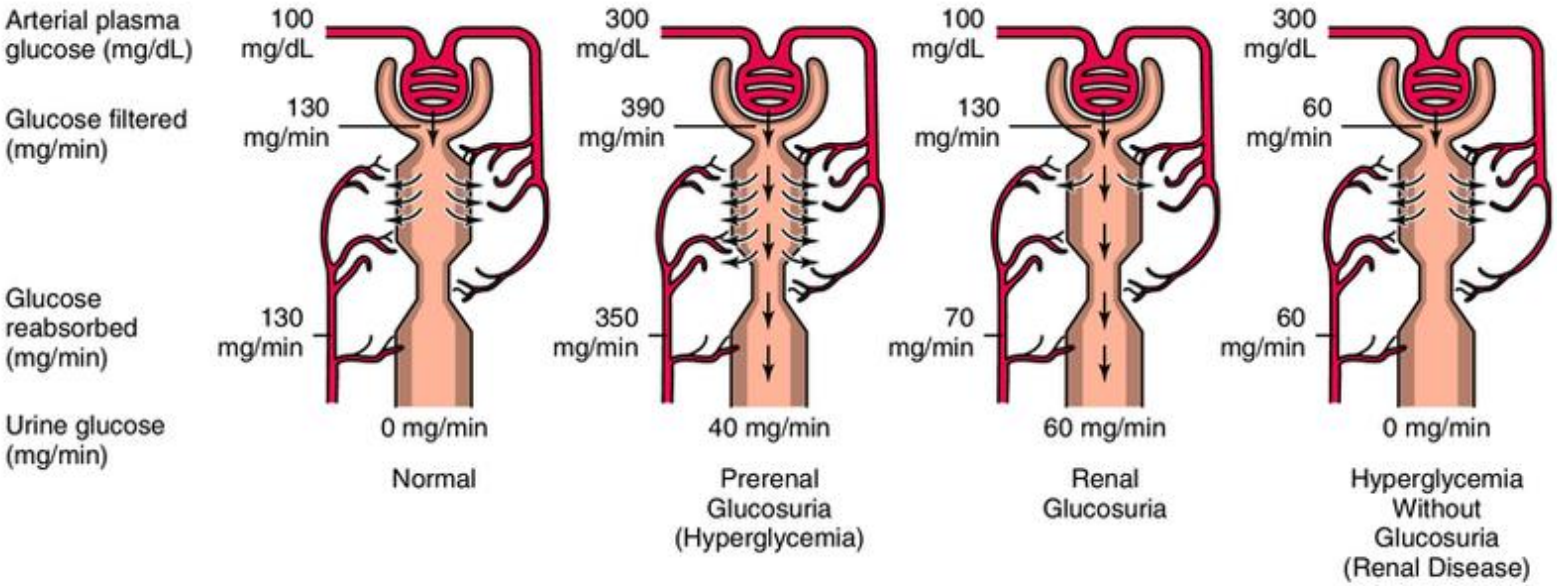
Figure 6-2. A schematic diagram comparing the filtration and reabsorption of glucose by proximal tubular cells normally and in conditions of hyperglycemia and renal tubular disease.



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Clinitest Tablets

Test for reducing substances

Detects sugars (except sucrose) and other reducing substances such as ascorbic acid, cysteine, and some drugs

Based on Benedict's copper reduction test:

- Reducing substances convert cupric sulfate to cuprous oxide
- Results in color change from blue to green to orange
- All necessary reagents contained in tablet

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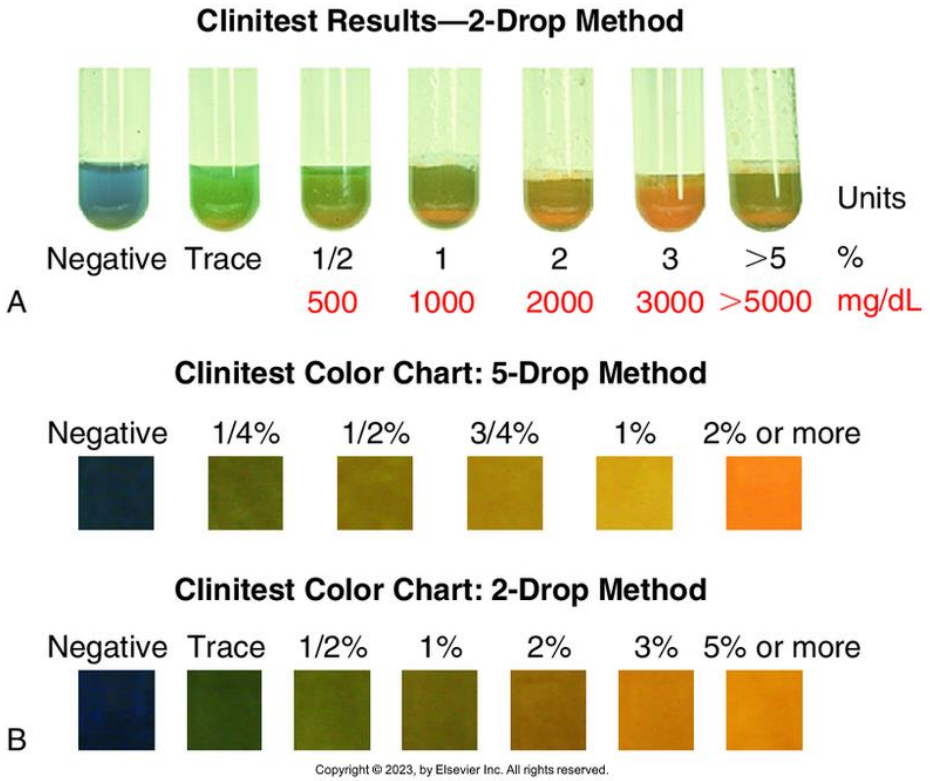

Figure 6-3. (A) A series of glucose standards analyzed using the Clinitest 2-drop method. Note that the tube with greater than 5000 mg/dL glucose has demonstrated the "pass-through" effect (i.e., after reaction, the mixture returns to a greenish color). (B) Clinitest color charts. Note the subtle differences between the 5-drop and 2-drop color charts. It is essential that reaction mixtures be compared with the proper color chart to obtain accurate results. *Do not use these color charts for diagnostic testing*.

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Ketones

Breakdown products from large amounts of fatty acids (more acetyl coenzyme A (CoA) formed than Krebs cycle can handle)

- Acetoacetate—principal form detected by reagent strip method
- Acetone
- β-Hydroxybutyrate—not detected by strip tests

Principle based on nitroprusside reaction

- In alkaline medium, acetoacetate reacts with sodium nitroprusside in pad
- Causes color change from beige to purple
- Some strips include glycine in pad, which allows acetone to be detected also

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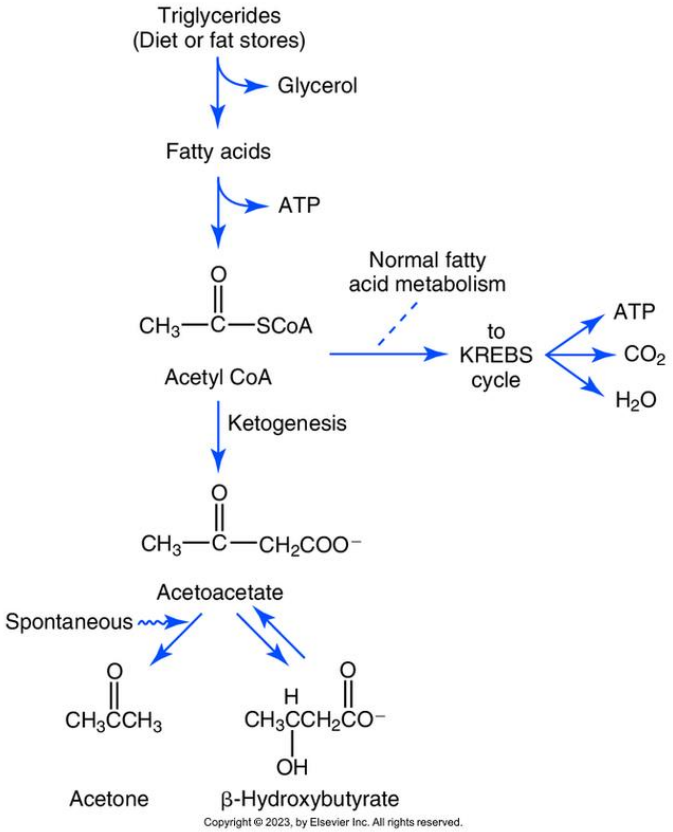
Figure 6-4. The formation of ketones from fatty acid metabolism. *ATP*, Adenosine triphosphate; *CoA*, coenzyme A; *SCoA*, succinyl coenzyme A.



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Bilirubin

Only direct (conjugated) bilirubin is water soluble and can be seen in urine in hepatic or posthepatic jaundice

Indirect bilirubin is not water soluble, so bound to albumin in plasma

Indirect bilirubin is filtered by the glomerulus and thus is too large to be observed in urine

Causes urine to be dark yellow to brown

Principle based on diazo reaction:

- Bilirubin reacts with a diazonium salt in pad to form azobilirubin, which is brown

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Urobilinogen

Best specimen is a 2-hour collection from 2 to 4 PM during the “alkaline tide”

- Urobilinogen excretion is enhanced in the slightly alkaline urine following a meal

Normally present in low amounts

Methodology depends on the brand of strip being used:

- Multistix uses classic Ehrlich’s reaction, which may also react with other substances
- Chemstrip and vChem strips use azocoupling reaction specific for urobilinogen

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Figure 6-5A. (A) A positive Acetest for ketones. (B) An Acetest color chart. *Do not use this color chart for diagnostic testing*.

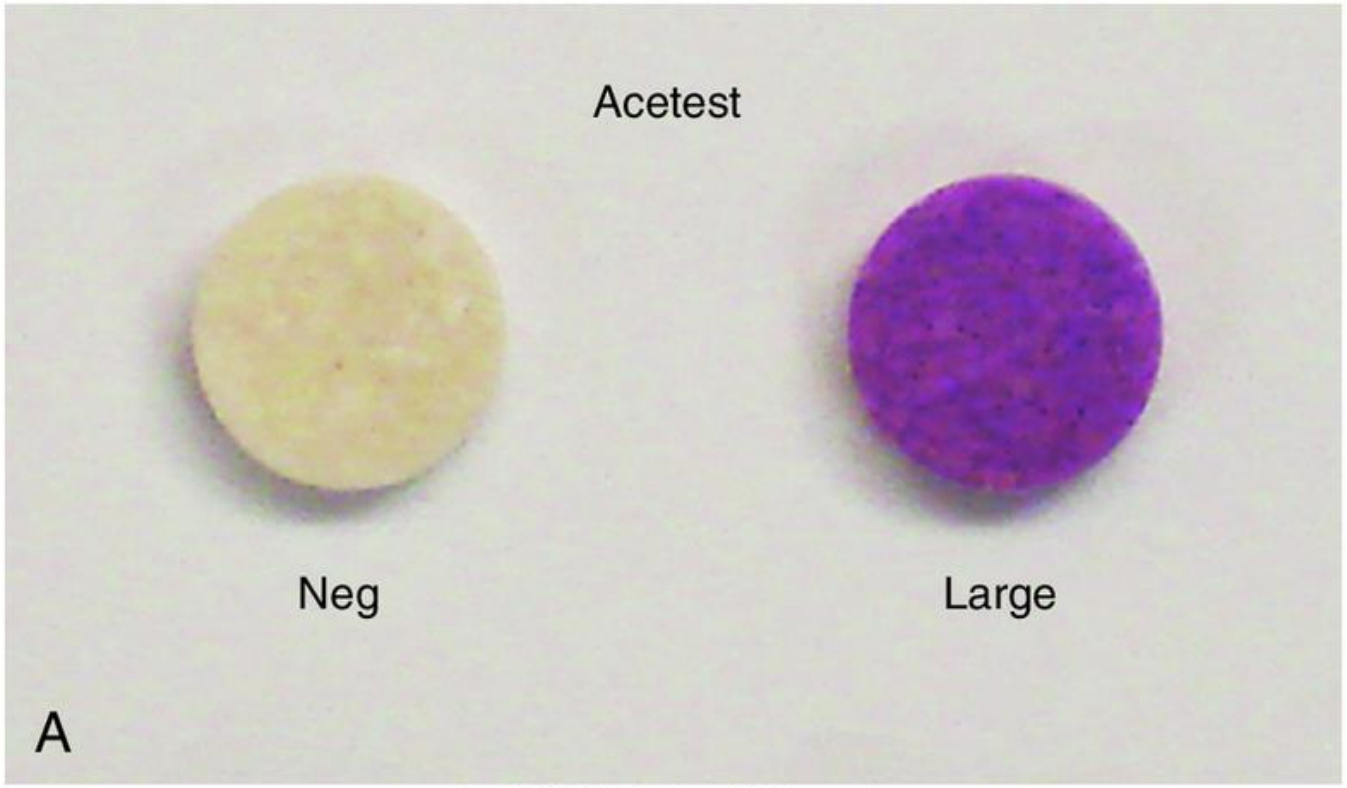

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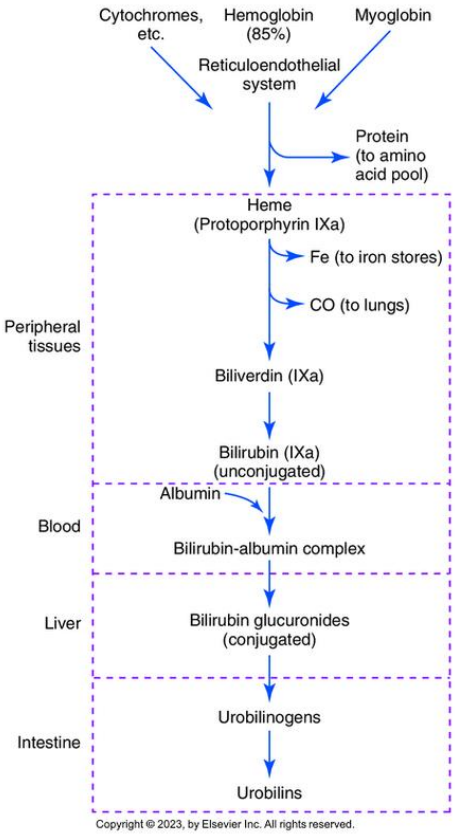
Figure 6-6. A schematic diagram of hemoglobin catabolism.



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Other Tests

Most tablet tests use same principles as strips, some with modifications (Acetest, Ictotest)

Acid-precipitation tests for protein, such as SSA, detect albumin as well as other proteins

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Figure 6-7. Bilirubin metabolism and alterations in normal metabolism caused by disease. (A) Normal bilirubin metabolism. (B) Prehepatic alteration of bilirubin metabolism. (C) Hepatic alteration of bilirubin metabolism. (D) Posthepatic alteration of bilirubin metabolism.

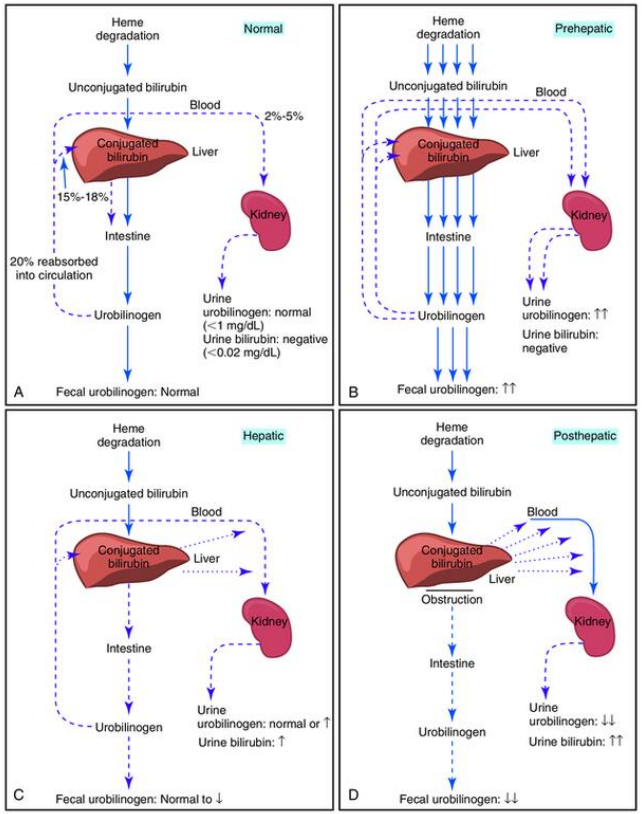

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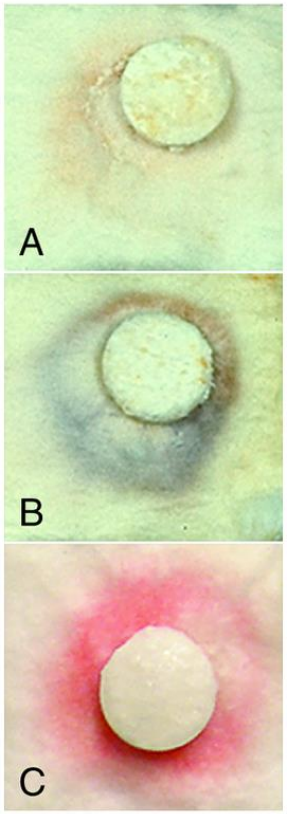
Figure 6-8. (A) A negative Ictotest. (B) A positive Ictotest for bilirubin. (C) A negative Ictotest showing an atypical color.



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