Supplemental File S1.

Methods of determination of historical parameters

Mucin

Mucin was reported not to dissolve in water but to swell in it, whereas it was noted to dissolve readily in weakly alkaline solutions. Mucin did not precipitate with mercuric chloride ($HgCl_2$), copper sulfate, or lead acetate, but that it formed a precipitate with acetic acid and basic lead acetate. To detect mucin, it was recommended to add twice the volume of 95% ethanol to the urine, after which the precipitate was neutralized with water. The characteristic features of such a precipitate are as follows: it does not become cloudy upon boiling; with acetic acid, it forms a precipitate insoluble in excess reagent but soluble in excess hydrochloric or nitric acid; it forms a precipitate with basic lead acetate; with the addition of Millon's reagent and boiling, it turns pink–red; and with sodium hydroxide (NaOH) and copper sulfate, it produces a biuret reaction [53].

Albumose

Albumose was detected similarly to protein (albumin) using Heller's test or ferricyanide reagent. To clear the urine, acetic acid was added to obtain a 2% CH_3COOH solution, and then, drops of potassium ferricyanide (1:20 solution) were added. In the presence of protein or albumose, the fluid became turbid. The turbidity caused by albumose disappears upon on heating [58].

Pus

For suspected pyuria, the addition of a small amount of acetic acid causes pus to settle as an opaque mass, resembling cream, jelly, or clay. An equal amount of potassium hydroxide dissolves the pus, forming a gelatinous mass with a foul odor, and ammonia is released as decomposition occurs. Pus bodies resemble leukocytes. The main difference is in quantity: normal urine contains 1–3 leukocytes per high-power field, whereas pus bodies may range from several dozen to 1000 per field, either scattered or in clusters [54].

Indican

Indican was detected in urine using two tests: Heller's test and Jaffé's test. In Heller's test, 5 mL of fuming HCl was mixed dropwise with the urine sample, and the resulting color change was observed. A pink color indicated a small amount of indican, whereas a red-violet or dark blue color that quickly changed to brownish-red or yellow indicated higher levels. A few drops of nitric acid (HNO_3) could be added to the mixture to accelerate the reaction.

In Jaffé's test, 10 mL of urine was mixed with a significant amount of hydrochloric acid, followed by the addition of calcium hypochlorite

($Ca(ClO)_2$), and shaking. Under the oxidizing action of chlorine, the indican in the urine decomposes, producing colored compounds that turn the urine red, violet, green, or blue, depending on the amount of indican present. During the filtration of such urine, a violet ring formed on the filter. Hammerstein recommended treating a mixture of urine with HCl and $Ca(ClO)_2$ with chloroform, which turned blue due to the indigo formed from indican [53].

Russo's reaction

Four drops of an aqueous methylene blue solution were added to 4–5 mL of urine. The urine that was turbid due to an excess of urates or phosphates was first filtered. If shaking the test tube produced a uniform mint or emerald green color, the result was considered positive. In a negative test, the color of the sample did not change. Even at the time of its introduction, the usefulness of this test was questioned. It is now known that the color change was related to the acidic pH of the urine [64,65].

Weisz (Weiss) urochromogen reaction

According to Weisz, pathological urine contains the pigment urochromogen, which oxidizes to urochrome. Urochromogen is entirely of endogenous origin. To detect urochromogen, ammonium sulfate was added to the urine to precipitate bilirubin, urobilin, and other pigments. After filtration, a solution of potassium permanganate (1 g/1 L of water) was added to the filtrate. In the presence of urochromogen, the urine turned yellow with a greenish tint [58].

Urea

Urea was initially measured using Liebig's method. Sulfates, phosphates, chlorides, and proteins were removed from the urine beforehand. The prepared urine was treated with mercuric nitrate, precipitating urea as a white solid. Mercuric nitrate was added until the sample, when mixed with a drop of sodium carbonate on a watch glass, developed an orange-yellow color. This color, which is dependent on the mercury oxide formed, appears only after all the urea in the urine has reacted with the mercuric nitrate [53].

Creatinine

Creatinine in urine was determined using Jaffé's method, which underwent many modifications but retained the same basic principles described above.

Uric Acid

Uric acid was typically measured using Ludwig's method. To 100 mL of urine, ammonium-magnesium salt and an ammoniacal silver solution were added, causing phosphoric acid to precipitate as ammonium-magnesium phosphate and uric acid as magnesium-silver urate. The filtered precipitate was boiled with potassium sulfide, which released silver sulfide, leaving uric acid in solution. The solution was separated

from the precipitate and evaporated to a small volume, after which hydrochloric acid was added over 24 hours to isolate the uric acid. Precipitates collected on glass wool were washed with carbon disulfide, dried, and weighed. If the filtrate was cloudy or brown, it was slightly acidified with hydrochloric acid and evaporated to dryness; the residue was dissolved in hot water, and potassium hydroxide was added dropwise until the uric acid dissolved. The solution was separated from silver sulfide, and uric acid was isolated again as previously described. Protein-containing urine was first deproteinated with concentrated table salt and acetic acid [58].

Chlorides

In the qualitative test, a few milliliters of urine acidified with nitric acid were treated with a silver nitrate solution (1:20). If an excess of chlorides were present, a white curd-like precipitate of silver chloride formed. In Neubauer's quantitative method, 10 mL of urine was mixed with 1 g of pure sodium carbonate, and the mixture was then evaporated to dryness and ignited to remove organic components. The resulting residue was dissolved in water, and 3–5 mL of nitric acid was added to neutralize the previously added sodium carbonate. The solution was subsequently filtered, and chlorides in the filtrate were precipitated according to Mohr's method. To the resulting filtrate, which should have a neutral or slightly alkaline pH, standardized silver nitrate was added from a burette until the previously added potassium chromate (a few drops) turned red. This indicated that all chlorides in the tested urine had precipitated as silver chloride and that the potassium chromate had been released as a brick-red precipitate of silver chromate. Potassium chromate served as an indicator, which only began to react with the silver ions once all chlorides in the urine had precipitated [53].

Sulfates

Sulfates were measured by adding hydrochloric acid to 100 mL of urine, followed by the addition of an excess of barium chloride ($BaCl_2$) solution, and then boiling the mixture. The resulting precipitate was collected on a pre-weighed filter, washed, dried, and weighed. The sulfate content was calculated from the weight of the barium sulfate precipitate, taking into account that 233 parts of $BaSO_4$ correspond to 98 parts of sulfuric acid, 80 parts of SO_3, or 32 parts of sulfur [53].

Phosphates

Phosphates were determined in urine by a titrimetric method. The procedure involved adding a solution of sodium acetate (100 g) and acetic acid (30 g) per liter of water to 50 mL of urine, together with a few drops of cochineal. The mixture was heated to boiling, and a uranyl solution was gradually added until the color changed from red to green, indicating the endpoint [67].

Acetoacetic Acid, Acetone, β-Hydroxybutyric Acid (Ketone Bodies)

In the past, acetoacetic acid was detected using Gerhardt's test, in which ferric chloride caused urine containing acetoacetic acid to turn violet-red. Acetone was detected using Legal's test, which involved adding a fresh alkaline solution of sodium nitroprusside to the urine; if acetone was present, the sample turned red. This reaction is still employed today in modern urine test strips. Detection of β-hydroxybutyric acid was performed using Black's test, which relied on the conversion of β-hydroxybutyric acid into acetoacetic acid by hydrogen peroxide in the presence of iron salts, resulting in a red coloration of the sample [63].

Blood Pigments (Hematuria)

Blood pigments in urine were historically detected using Adler's benzidine test. In this test, an equal volume of glacial acetic acid was added to 10–15 mL of urine, and the mixture was extracted with ether. To the ether solution, a solution of alcohol saturated with benzidine and a few drops of hydrogen peroxide were added. If blood was present, the solution turned green, indicating a positive result [62].

Bile Pigments (Bilirubin and Urobilinogen)

Bile pigments historically included cholepyrin (from the 1880s referred to as bilirubin), biliverdin, and bilifein, athough these were not distinguished on test requests or results, functioning under a common name. Cholepyrin was detected by slowly adding nitric acid along the walls of a vessel containing approximately 20 mL of urine. If cholepyrin was present, a ring formed at the interface between the acid and the urine, showing rapid color changes from green to blue, violet, red, and finally yellow. If the test was negative, biliverdin was tested next. Urinary pigments were precipitated with lead acetate, and the washed, dried precipitate was treated with a few drops of sulfuric acid. A greenish coloration of the filtrate indicated the presence of biliverdin. Bilifein was detected by adding an equal volume of hydrochloric acid to 20 mL of urine in a white glass vessel, mixing, and then adding a few drops of nitric acid. The mixture was observed under light while being continuously stirred. If bilifein was present, a color change occurred similar to that of cholepyrin, but ultimately the color remained green [68].

In 1892, Paul Ehrlich developed a method for detecting bilirubin. Bilirubin reacted with diazo sulfanilic acid (Ehrlich's diazo reagent) to form the colored derivative azobilirubin, with the intensity of the red coloration proportional to the amount of bilirubin. The reaction was nonspecific, primarily reacting with the pyrrole group [58].