Laboratory-Prepared Quality Control Solution for Urine Test Strips

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DOI: https://doi.org/10.51244/IJRSI.2024.11150064P

Received: 10 November 2024; Accepted: 13 November 2024; Published: 21 December 2024

ABSTRACT

A urinalysis test consists of three distinct examinations: physical, chemical, and microscopic. The various chemical tests are performed with a urine strip. A urine strip is a plastic dipstick with absorbent square pads embedded with chemicals that represent certain reactions. A quality control scheme should ensure that the urine strips are functioning properly by detecting the positives in various grades. A proposed laboratory-prepared control solution is a mixture of glucose powder, acetone, human serum, hemolyzed human red cells, and normal saline solution. This solution serves as a positive control to test the accuracy of urine strips for sugar, protein, pH, blood, and density. The triplicate daily test results in these parameters were consistent and showed no significant difference. This laboratory-prepared control solution is equivalent to a positive control level 2 and stable until 15 days when properly dispensed in sterile capped tubes and kept at a refrigerated temperature.

Keywords: Urine test strip, control solution, parameter, urinalysis, quality control

INTRODUCTION

The analysis of urine is a basic test in the diagnostics for most kidney diseases and systemic disorders. Appreciable information may be obtained from a urine test as disease processes invariably manifest urine abnormalities. A urinalysis test consists of three distinct examinations: physical, chemical, and microscopic. Urine physical examination takes notice of specimen color, clarity, and volume [1]. The microscopic examination of urine includes the quantification and qualification of cells, crystals, casts, and microorganisms in the sediments after standard centrifugation [2]. The various chemical tests determine the urine pH (a measure of acidity or alkalinity), proteins, density, glucose, urobilinogen, bilirubin, ketone bodies, leukocyte esterase, and nitrites with a multi-stick [3].

A urine strip is a plastic dipstick, about 4 - 6 mm wide and 11 - 12 cm long, with absorbent square pads embedded with chemicals that represent certain reactions [1]. The urine strip is dipped into a freshly voided urine sample and observed for color change if a substance is present at an abnormal level. Chemical reactions take place in the square pads resulting in a color change which may be assessed visually [3]. An acceptable sample for processing is fresh, well-mixed, uncentrifuged urine [2]. The quality of the urine sample and the patient preparation are essential in producing correct results [4]. The specimen is kept at room temperature and must be tested within an hour after voiding to prevent erroneous results caused by changes that may occur in unpreserved urine [2]. The urine specimen can be tested after pouring an aliquot into a centrifuge tube. A urine strip is briefly dipped into the urine sample wetting all the test pads. The time required before color development varies with the test parameter and must be followed to obtain reproducible and reliable results. The urine strip should be properly oriented and read close to the color chart in a well-lit area [6]. The color changes in the chemical pads are noted visually and recorded.

Urine strips fall into the Clinical Laboratory Improvement Amendments (CLIA)-waived category and are generally very reliable [8]. Quality control testing of reagent strips may be overlooked by laboratories

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI | Volume XI Issue XV November 2024 | Special Issue on Public Health

thinking that urine strip results are dependable and highly reproducible. As long as the urine strip expiration is not reached and the pads in the strips are not discolored, medical technologists would usually trust the use of these strips. However, there is no guarantee that it will be error-free. Using urine strips is convenient, however false-positive and false-negative results can occur [4]. If the canister of urine strips is not recapped and left open for some time, the urine strips are exposed to air and humidity leading to reagent degradation and false results [5]. Similar to other laboratory determinations, quality control of the chemical test strips should be regularly and strictly practiced. All clinical laboratory tests, including CLIA-waived tests, should follow a routine Quality Control program as per other moderate and high complexity tests according to the College of America Pathologists Laboratory Accreditation Program [7]. A quality control scheme should ensure that the urine strips are functioning properly by detecting positives (Level 2 control) or pathologic results, not only the negatives (Level 1 control). In testing any kind of patient sample for indications of disease, reliable and stable pathologic controls must be used to validate urine strip performance and ensure accurate patient diagnosis. Furthermore, the laboratory is responsible for having control procedures that monitor the accuracy and precision of the complete analytical process [7].

Commercial or laboratory-prepared materials can serve as acceptable pathologic or positive controls. Regardless of the control material used, care must be taken to ensure that analyte values are within the critical detection levels for each parameter [9]. A negative control test employing distilled water is not enough as only the absence of an analyte is evaluated. A positive or pathologic control to test the reactivity and sensitivity of the urine strip is needed. Considering the requirements and care for multiple parameters on the urine strip, most laboratories purchase positive or pathologic control materials rather than prepare them. Remote and small laboratories, however, are discouraged by the cost and low practicability of commercial pathologic or positive controls.

This study is a comprehensive review of the chemical parameters of the urine strips with an emphasis on the importance of performing quality control using a laboratory-prepared positive control solution at the pathologic level. The sole objective is to promote the preparation and use of an internal positive control (Level 2) solution with materials that are easily accessible and cost-efficient and may be adopted by even primary and free-standing laboratories in far-flung areas.

METHODOLOGY

A. Research Design

A laboratory-prepared positive control solution or pathologic control for urine strips was best presented after a thorough trial and error in the composition of the solution and testing. The control solution was carefully mixed and subjected to daily testing while observing the optimum storage for use. The lab-prepared control solution was tested using the urine strip parameters for sugar, protein, pH, blood, and density in triplicate using three brands of urine strips in two separate cycles from January – February 2023 with permission at the Metro Clinical Laboratory.

B. Materials

The Laboratory-prepared positive control solution is a blend of glucose powder, acetone, human serum, hemolyzed human red cells, and normal saline solution (NSS). The control solution was dispensed in 5 mL aliquot in sterile capped tubes. Three brands of urine strips (designated as urine strips A, B, and C) with multiple parameters, similar sensitivity, and grading were used to test the positivity.

C. Process

In a clean and dry flask, 2 grams of glucose powder were dissolved in 50 mL of NSS. 5 mL of pooled human serum and 1 mL of hemolyzed blood were added. The solution was mixed by swirling on a flat benchtop for 5 minutes. 500 uL of acetone was added drop by drop while swirling. The total volume of the control solution was brought to 100 mL by adding NSS.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI | Volume XI Issue XV November 2024 | Special Issue on Public Health

Human serum was prepared by pooling from normal non-infectious samples in the laboratory. An acceptable pooled serum for the control solution is clear in transparency, pale yellow, and was tested negative for transfusion-transmissible infections (HIV, HBsAg, HCV, malaria, and RPR). Hemolyzed blood was prepared from a blood sample in EDTA. 250 uL of blood was placed in 1 mL of distilled water and thoroughly mixed by inversion. Pooled serum samples and blood samples in EDTA were requested with due permission from the Metro Clinical Laboratory.

The laboratory-prepared positive control solution was dispensed in 5 mL aliquot on 20 dry sterile capped tubes. This control solution batch is kept at a refrigerated temperature. The date of preparation was noted. The researcher takes out one tube for urine strip control testing each day. Used tubes were not returned to the refrigerator nor reused.

In Figure 1, the urine strips were immersed briefly in the lab-prepared positive control solution and compared with the color chart. The presence of chemicals caused the color change in the chemical square pads of the urine strips and the intensity of color indicates their concentration. The materials employed to concoct the lab-prepared control solution are also shown.

D. Data Gathering Procedure

Testing of the lab-prepared control solution was performed on a well-lit safety cabinet with a clean and dry work area, free from fumes and volatile chemicals. The canister of urine strips, lab-prepared control solution, test tube rack, distilled water, and absorbent paper were prepared. The researcher first performed a negative control test using distilled water on the urine strips and observed the absence of any color change in the urine strip chemical pads. The entire canister was discarded and a new batch was opened for any unacceptable result or signs of deterioration in the urine strip.

The researcher took out one control tube for each day of testing. This tube was allowed to equilibrate to room temperature for 15-30 minutes before use. The testing was done in triplicate using 3 various brands of urine strips. The control solution tube was held firmly and tilted. A urine test strip was briefly dipped while fully immersed in the control solution to saturate the chemical pads. The excess control solution was drained from the strip by rimming on the tube and by placing the strip edge on an absorbent paper. The urine strip was properly oriented and read close to the color chart. Each chemical pad was read at the manufacturer's designated time printed on the canister label to obtain reproducible and reliable results. The recommended test strip read time for glucose was 30 seconds, density in 45 seconds, and blood, pH, and protein in 60 seconds [10]. The color changes in the chemical pads were evaluated visually and recorded.

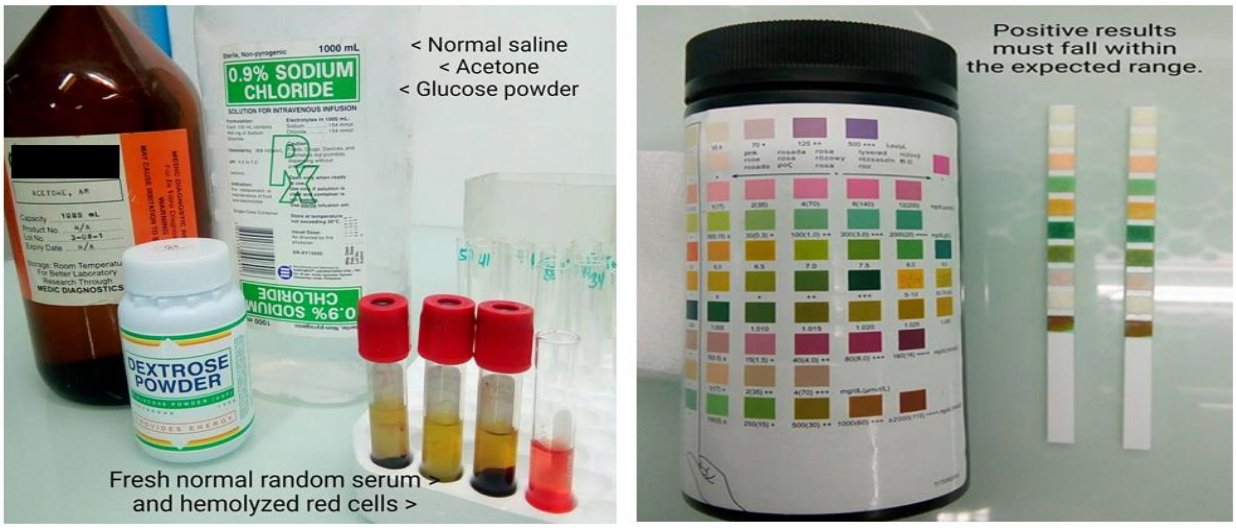

FIGURE 1. THE URINE STRIP AGAINST THE COLOR CHART AND THE COMPOSITION OF THE LAB-PREPARED POSITIVE CONTROL (LEVEL 2) SOLUTION

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI | Volume XI Issue XV November 2024 | Special Issue on Public Health

TABLE I. DAILY MEAN POSITIVITY RESULTS OF THE LABORATORY-PREPARED CONTROL SOLUTION

Parameter	Glucose	Protein	Blood	pH	Density
Expected results	3+ - 4+	2+ - 3+	2+ - 3+	6.0	1.010 – 1.015
Day	Results				
1	4+	3+	3+	6.0	1.015
2	4+	3+	3+	6.0	1.015
3	4+	3+	3+	6.0	1.015
4	4+	3+	3+	6.0	1.015
5	4+	3+	3+	6.0	1.015
6	4+	3+	3+	6.0	1.015
7	4+	2+	3+	6.0	1.015
8	4+	2+	3+	6.0	1.015
9	4+	3+	2+	6.0	1.015
10	4+	2+	2+	6.0	1.015
11	4+	3+	2+	6.0	1.015
12	4+	2+	2+	6.0	1.010
13	4+	2+	2+	6.0	1.010
14	4+	2+	2+	6.0	1.010
15	4+	2+	2+	6.0	1.010

RESULTS

A tube of laboratory-prepared control solution was tested for positivity with multiple parameters of urine strips. The results were duly recorded in grades for glucose, protein, and blood, and numerically for pH and density. The testing for the lab-prepared control solution was performed daily for 15 days.

In Table I, the chemical results in the urine test strips were consistently replicated in most parameters up to the last day of testing. The mean positive results were noted in sugar: 4+, density: 1.010 to 1.015, blood: 3+, pH: 6.0, and protein: 2+ to 3+.

The sugar test in the urine strip detects the presence of glucose in the lab-prepared positive control solution. The principle is based on the glucose oxidase reaction wherein gluconic acid and hydrogen peroxide are formed from the oxidation of glucose by the enzyme. The peroxidase catalyzes the reaction of hydrogen peroxide with the chromogen giving the observable color change in the square reaction pad [11]. The color varies from light green to brown corresponding to the following range of glucose concentrations in mg/dL: negative, trace (100), 1+ (250), 2+ (500), 3+ (1000), and 4+ (>2,000) [13]. The control solution contains 2 grams in 100 mL or 2,000 mg/dL corresponding to a 3+ to 4+ result in the urine strip.

Urine strip blood detection is based on free hemoglobin peroxidase activity which catalyzes the liberation of oxygen from peroxide [13]. A benzidine compound is oxidized to a colored product ranging from orange to green. From a negative yellow color, the positivity produces a green color and flecked if the red blood cells are intact. The color fields correspond to the hemoglobin in an estimated number of red blood cells per uL in the following values: negative, trace (5), 1+ (10), 2+ (50), and 3+ (>250) [12]. A component of the lab-prepared positive control solution is 1 mL of hemolyzed human blood. The free hemoglobin gives a square reaction pad reaction between 2+ to 3+.

The pH test reaction pad uses methyl red and bromthymol blue indicators which provide pH readings from 5.0 to 8.5. The color of the test pad changes from orange to green to blue [12]. The components in the lab-

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI | Volume XI Issue XV November 2024 | Special Issue on Public Health

prepared control solution when properly mixed and tested with a pH meter on various days give a result of pH 6. This pH result was consistently replicated using the urine strips.

TABLE 2. VARIATION IN THE LAB-PREPARED CONTROL SOLUTION RESULTS

Parameter	Average	Variance	F crit	Computed F	Decision	Interpretation
Glucose	4.0	0	3.2199	0	Accept Ho	Not significant
Protein	2.53	0.267	3.2199	6.66E-15	Accept Ho	Not significant
Blood	2.53	0.267	3.2199	6.66E-15	Accept Ho	Not significant
pH	6.0	0	3.2199	0	Accept Ho	Not significant
Density	1.0137	0.00000524	3.2199	1.29E-14	Accept Ho	Not significant

The protein test in the urine strip operates on the principle of "protein error of indicators" which is the ability of albumin protein to alter the color of some acid-base indicators without altering the pH of the solution [11]. The albumin present in the lab-prepared positive control solution coming from the pooled serum reacts with the tetrabromophenol blue indicator resulting in a yellow to green-blue color change [12]. The color fields in the urine strip correspond to the following range of albumin concentrations in mg/dL: negative, trace (15), 1+ (30), 2+ (100), 3+ (300), and 4+ (2,000) [11]. The lab-prepared positive control solution range is expected between 2+ to 3+, depending on the concentration of albumin in the pooled serum used.

The urine density test is the specific gravity indicating the ion concentration of the liquid sample. The urine strip reaction pad contains bromthymol blue, sodium hydroxide, and polymethylvinyl ether/maleic anhydride. Increasing ion concentrations cause a color change from blue-green to yellow [14]. The test allows the determination of specimen density between 1.000 and 1.030. The lab-prepared positive control solution has been tested with a refractometer on various days and gives a range between 1.010 to 1.015. This density was consistent with the urine strip test results.

In Table II, the daily triplicate results in testing the lab-prepared control solution for the parameters were computed and compared. The positive results were consistent across the triplicate urine strips. In the parameters of glucose, protein, blood, pH, and density, there were no significant statistical differences in the results compelling the researcher to accept the null hypothesis.

DISCUSSION AND CONCLUSION

The laboratory-prepared positive control solution contains components detectable in the various parameters of the urine strips. Quality Control surveys in urinalysis by the reagent strips between manual and automated methods indicated differences because the reagent strips and instruments have different characteristics [15]. The observer must check the range of the analyte per grade expressed in plusses. Automated methods on the other hand make use of test strip technology that enables a more efficient quantitative analysis of chemicals and cells in the urine. The complementary metal oxide semiconductor-based strip reader in combination with reflectance and classical dye-binding obtains very sensitive readings [16].

Some middle-to-low concentration positive quality control liquid for urine analysis have been patented. The positive quality control liquid comprises a buffer solution (pH 4.5 - 8.5), surfactant, protein and carbohydrate protectants, anhydrous glucose, bovine serum albumin, bovine hemoglobin, esterase, sodium ethyl acetoacetate or ethyl acetoacetate, and a direct bilirubin or naphthylamine salt [17]. Several other products of this kind utilize purchased materials which is not cost-effective.

To strictly pursue quality control and optimal results for multiple parameters urine strips, laboratories may conveniently purchase positive or pathologic control materials rather than prepare them. However, small or remote laboratories may find the cost discouraging. Not to mention the short shelf life of opened control solutions that may only last for a month [15].

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI | Volume XI Issue XV November 2024 | Special Issue on Public Health

The recommended storage of the laboratory-prepared control solution has been established at 15 days wherein the 5 mL aliquot in sterile capped tubes remained stable and uncontaminated. Refractometer testing, pH meter testing, and spectrophotometric tests for sugar and protein ensured the stability of analytes in the control solution without any significant change until after 15 days. At this time the control solution remained clear at refrigerated temperature. Random bacteriologic testing of 3 tubes of the lab-prepared control solution batch, when plated in enrichment media on the 15th day, did not show any growth. Note that 500 uL of acetone was added to deter the growth of microorganisms in the lab-prepared control solution which contains serum and blood, conducive for bacterial growth.

Since the proposed lab-prepared control solution is not yet patented and duly validated by health regulatory authorities, the researcher highly recommends further studies and refinement of the preparation methods, including the proper validation of such products for routine purposes. The researcher has been utilizing and improving this lab-prepared control solution for years.

In conclusion, the clinical laboratory can prepare its positive control solution, equivalent to Level 2 control, using a mixture of readily accessible materials like normal saline solution, hemolyzed blood, human serum, glucose powder, and acetone. This lab-prepared control solution can be used for efficiently monitoring the precision and reactivity of urine test strips for chemical testing. Dispensing the lab-prepared control solution aliquot in sterile capped tubes and keeping them in the refrigerator preserve the stability of contents for 15 days.

Disclosure statement

The researcher has no conflict of interest to disclose.

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ISSN No. 2321-2705 | DOI: 10.51244/IJRSI | Volume XI Issue XV November 2024 | Special Issue on Public Health

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