Semiquantitative Determination of Urinary Glucose : Comparison of Home-Made Strip and Routine Tests

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Abstract

Glucose oxidase paper strips for semiquantitive determination of glucose in urine are commercially available but details of their preparation are not published. The purpose of this study was to produce such strips, using a coupled enzyme glucose oxidase-peroxidase reaction which would yield purple color with ortho-tolidine, and safranin upon dipping into urine containing glucose. In this present study, without any special equipment, special humidity and temperature control, the new reagent strip named R-strip could be successfully prepared in the atmospheric conditions of Thailand. R-strips were evaluated against random urine added with various amounts of glucose in comparison with a commercial strip (T-strip), Benedict's test, and a commercial tablet (C-tablet), routinely used in laboratories. The developed strips were found to be as specific as T-strip and more sensitive than other tests.

Key word : Semiquantitative Test Strip, Urinary Glucose, Glucose Oxidase Test

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It has been known for decades that glucose in urine can be tested by its reducing ability in which Benedict's test based on this property was the most widely used. A practical commercial method is now generally available, in which copper sulfate and alkali are combined in a dry tablet, liberating heat when dissolved in the urine allowing the reaction to proceed. This tablet is called C-tablet. However, the test is not specific for glucose or even for reducing sugars. The first glucose oxidase method for determination of glucose in blood and urine, described by Froesh and Renold in 1956[1], gave clinical biochemists the first specific method for determination of glucose. Later in the year, the use of a coupled enzyme system was suggested, in which hydrogen peroxide formed in the glucose oxidase

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reaction, is catalyzed by peroxidase, forming color with a suitable chromogenic oxygen acceptor in direct proportion to glucose concentration. When glucose oxidase, peroxidase, chromogenic oxygen acceptor, were impregnated in the filter paper in proper concentrations, it offered semiquantitative determination of glucose in urine[2]. The usefulness of glucose oxidase strips is also provided for cerebrospinal fluid and pulmonary aspiration[3-5]. Investigations and comparison of variable test-strips from several companies have recently led to the avaibility of more accurate and convenient urinary glucose measurement[6-8]. However the methods for preparation in detail have not been described. It was the aim of this study to assess the efficacy of the double enzymes impregnated filter paper strip (R-strip) for semiquantitative enzymatic determination of glucose in urine, which was developed in the clinical pathology laboratory, Ramathibodi Hospital. Comparison of this new strip with a commercial strip (T-strip) has been used for routine analysis of glycosuria, Benedict's test an original reduction test and C-tablet a convenient commercial test for mellituria or any non-sugar reducing substances in urine were performed.

MATERIAL AND METHOD

1. Preparation of glucose oxidase paper strip

Optimal concentration of glucose oxidase, peroxidase, O-tolidine were readily found by varying concentrations of one gradient in the presence of optimal or excess concentration of the other two. Final ingradients were recommended : glucose oxidase type II with activity of 20,300 units per gram (Sigma Chemical Co.) 12,000 units, Horseradish peroxidase type II with activity of 14.5 purpurogallin units per mg (Sigma Chemical Co.) 7,500 units O-tolidine 0.44 g, Safranin (saturated) 2.5 ml. The volume was made to 100 ml with 0.8 mol/l EDTA-Tris buffer pH 5.1. Whatman chromatography paper of 0.3 mm thickness was gradually soaked into the solution, after saturation, the paper was taken up and rapidly dried at room temperature in the dark. The dried paper was cut into strips of 6 mm x 12 mm. The strips were kept in dark bottles in a desiccator when not in use. In the process of evaluation, test strips were dipped into the urine about half of their lengths. The intensity of the purple color read 10 seconds to 30 seconds after dipping was graded as +, ++, +++, ++++.

2. Evaluation of the developed strip in comparison with T-strip, C-tablet and Bendict's test

Fresh random urine samples which were negative for glucose by T-strips were collected. All negative urine samples were subjected to Benedict's test. Urine samples that were negative with these two methods were judged to be free of a significant amount of reducing substance. Stock standard glucose solutions were added to the negative urine making final concentrations of 0.05, 0.10, 0.25, 0.5, 1.0 and 2.0 g/dl respectively. One hundred specimens for each concentration of glucose, were used in the study.

Urine samples with specified glucose concentrations were prepared each day then coded and given as unknown for testing of glucose using Benedict's test, C-tablets, T-strips, and developed glucose oxidase strips (R-strip). The procedure was carried out by two experienced laboratory technologists. The analysis for glucose concentrations using T-strips and R-strips were also performed by two laboratory assistant students (who were considered to be inexperienced personnel.) Direction sheets and color charts were produced and given to all investigators.

RESULTS

On a series of 530 random urine specimens which were negative with T-strip, 72 samples produced trace positive with Benedict's test. None of these 72 samples were positive with R-strip. The remaining 458 specimens were judged to be negative in basal test, and they were used for further evaluation. The evaluation of R-strip, T-strip, Benedict's test, and C-tablet by different investigators, in 700 samples with varying glucose levels are shown in Tables 1, 2, 3, 4 respectively. Comparison between per cent of correct results from these four tests at expected concentrations of glucose added are shown in Fig. 1, using average values from two experienced operators. To analyse by T-strip the most even distribution of correct reading was produced in all range of glucose level.

Distribution of correct and incorrect results of R-strip and T-strip are shown in Fig. 2 and 3. Distribution of correct and incorrect readings of Benedict's test and C-tablet are demonstrated in Fig. 4 and 5. A correct reading means the reading that falls within the range of glucose level in the charts. One or more than one step errors are based

Table 1. Results with R-strip on urines with and without added glucose.

Reading		Negative				1+				2+				3+				4+			
Expected glucose level		0%				0.1% or less				0.25%				0.5%				1% or more			
		Investigator																			
% Glucose Added	No. of test	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	100	100	100	100	100																
0.05	100	1	1	1	1	89	87	87	86	10	12	12	13								
0.10	100					65	68	66	64	30	27	32	32	5	5	2	4				
0.25	100					14	7	13	16	68	70	72	68	18	23	15	16				
0.50	100					11	11	10	13	19	21	17	18	64	61	65	62	6	7	8	7
1.00	100					4	3	5	4	19	15	20	17	24	25	25	32	53	57	50	47
2.00	100					0	1	1	2	6	7	6	10	27	27	33	26	67	65	60	62

1 and 2 = Reported from trained investigators.
3 and 4 = Reported from untrained investigators.

Table 2. Results with T-strip on urines with and without added glucose.

Reading		Negative				Light				Medium				Dark			
Expected glucose level		0%				0.25% or less				0.25-0.5%				0.5% or more			
		Investigator															
% Glucose Added	No. of test	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	100	100	100	100	100												
0.05	100	12	10	12	12	84	85	85	82	4	5	3	6				
0.10	100	4	5	5	5	77	82	81	84	19	13	14	11				
0.25	100					39	31	42	45	55	60	53	49	6	9	5	6
0.50	100					12	13	20	19	40	43	44	47	48	44	36	34
1.00	100					4	6	7	8	36	32	38	42	60	62	55	50
2.00	100					2	3	3	2	15	16	23	28	83	81	74	70

1 and 2 = Reported from trained investigators.
3 and 4 = Reported from untrained investigators.

Table 3. Results with Benedict's test on urines with and without added glucose.

Reading		Negative		Trace		+		++		+++		++++	
Expected glucose level		0%		0.1-0.25%		0.5%		1%		1.5%		2%	
		Investigator											
% Glucose Added	No. of test	1	2	1	2	1	2	1	2	1	2	1	2
0	100	100	100										
0.05	100	20	21	79	78	1	1						
0.10	100			73	70	26	29	1	1				
0.25	100			16	21	71	67	13	12				
0.50	100					46	40	47	54	7	6		
1.00	100					7	2	63	65	18	24	12	9
2.00	100							1	0	29	34	70	66

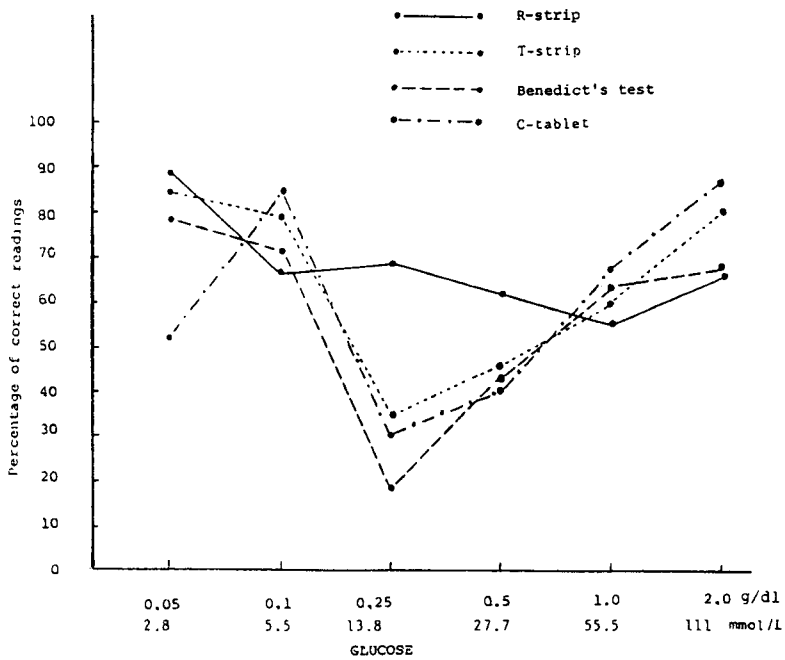

Fig. 1. Comparison of correct reading obtained from four tests at various concentrations of glucose in urine.

on the difference from expected reading in steps, as indicated in the charts. If more than one step error is justified to be a critical error, incorrect reading does not exceed 20 per cent in all tests at specified glucose added (Fig. 2, 3, 4, 5) except at 1 g per cent glucose level read by inexperienced investigators. Delaying the reading to 30 seconds when the results are +++ or more making more than one step error in reading decreased. The one step underreading at 0.05 per cent glucose, meaning false negative, occurred in only 1 per cent with R-strip. When the results are read at 10 seconds after dipping in the urine following T-strip manufacturer's instruction its sensitivity at 0.05 g per cent of glucose is much

Table 4. Results with C-tablet on urines with and without added glucose.

Reading		Negative		Trace		1+		2+		3+		4+	
Expected glucose level		0%		0.25%		0.5%		0.75%		1%		2%	
		Investigator											
% Glucose Added	No. of test	1	2	1	2	1	2	1	2	1	2	1	2
0	100	100	100										
0.05	100	49	48	51	52								
0.10	100	10	10	86	84	4	6						
0.25	100			32	29	64	66	4	5				
0.50	100			5	6	42	39	47	49	5	6	1	0
1.00	100					1	1	10	11	67	70	22	18
2.00	100									10	14	90	86

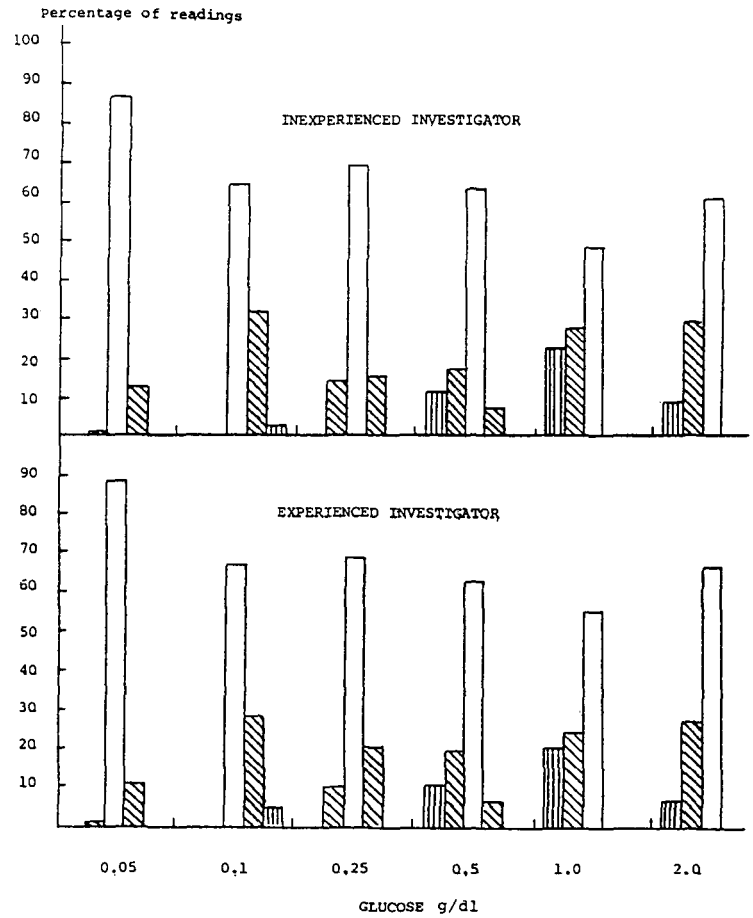

Fig. 2. Demonstration of correct reading of R-strip in comparison with various incorrect readings.
☐ correct reading ▨ one step error in reading ▥ more than one step error in reading.
Left hand side of correct reading = underreading
Right hand side of correct reading = overreading

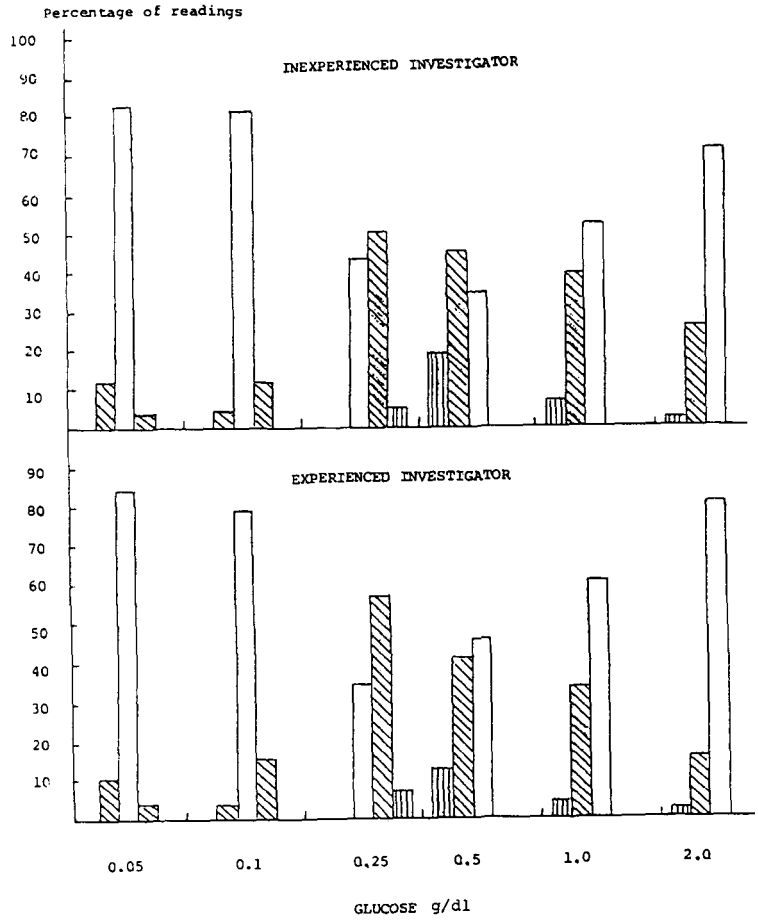

Fig. 3. Demonstration of correct reading of T-strip in comparison with various incorrect readings. ☐ correct reading ▨ one step error in reading ▥ more than one step error in reading. Left hand side of correct reading = underreading Right hand side of correct reading = overreading

poorer than R-strip (Table 2). If the reading was delayed to 30 seconds, the purple color developed in 8 samples and its sensitivity is much closer to R-strip.

R-strip showed mini-ascending chromatography in the average of 40 samples from 700 samples overall. In these cases the stronger reaction occurred above the area where the strips were dipped. The reading at the upper area of 20-25 samples were correct, equal to the glucose concentration, which showed a stronger reaction than the reading from the dipping area.

DISCUSSION

This study demonstrated that glucoseoxidase-peroxidase filter paper strip for semiquantitative determination of glucose in urine, can be successfully prepared in a laboratory with minimum equipment. EDTA-Tris buffer pH 5.0-5.5 is the buffer of choice because it has many desirable qualities. It may be due to the ability of EDTA to chelate metallic ions which can markedly inhibit the action of glucose oxidase[9]. Tris is known to be bacteriostatic and this property may improve the stability of the strips.

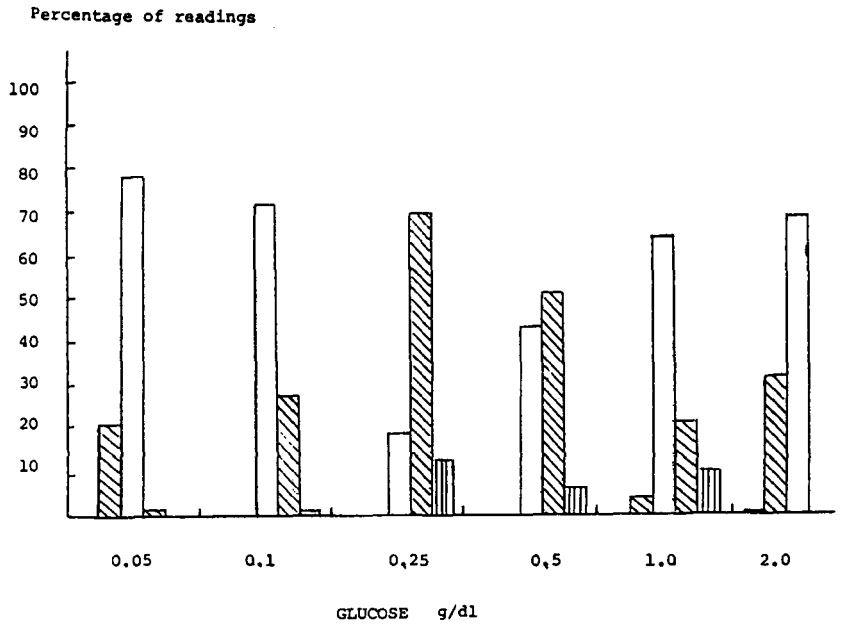

Fig. 4. Demonstration of correct reading of Benedict's test in comparison with various incorrect readings. ☐ correct reading ▨ one step error in reading ▥ more than one step error in reading. Left hand side of correct reading = underreading Right hand side of correct reading = overreading

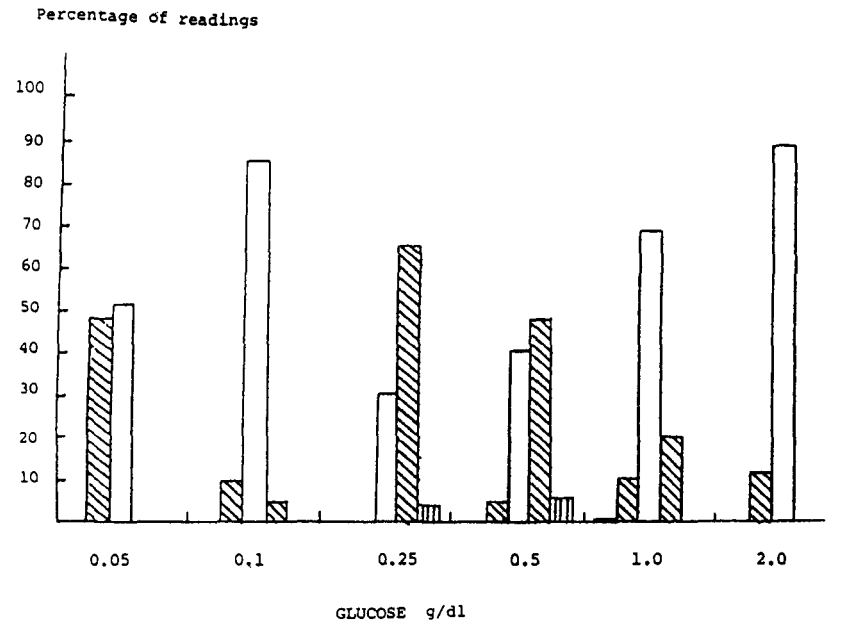

Fig. 5. Demonstration of correct reading of C-tablet in comparison with various incorrect readings. ☐ correct reading ▨ one step error in reading ▥ more than one step error in reading. Left hand side of correct reading = underreading Right hand side of correct reading = overreading

The improved enzyme strip is very sensitive for the detection of glucose as about 0.05 per cent (2.8 mmol/l) of glucose in urine will give a positive reaction. It can be seen in Table 1 that 99 in 100 urine samples containing 0.05 per cent glucose gave a positive reaction with R-strip while T-strip was definitely less sensitive to detect glucose at the added concentration of 0.05 and 0.1 per cent. The manufacturer of T-strip claimed the sensitivity of only 0.1 g per cent (5 mmol/l) of glucose. The apparent better sensitivity of R-strip could be either due to the freshness of this preparation or some significant improvement in methodology. As it is impossible either to test with a freshly prepared T-strip or to know its exact composition, the reason for the better sensitivity of R-strip could only be speculated. Age, exposure to moisture, and light reduced the sensitivity of the strip while pH, temperature, of urine were not significant[2,10]. Poorer sensitivity of the glucose in Benedict's and C-tablet reconfirmed other reports[11,12].

It could be seen that 13.58 per cent of the urine specimens which gave a negative reaction with the glucose oxidase test T-strip were positive with Benedict's test, while none of the 72 persons were positive by R-strip. All urine sepcimens which were negative for the basal test were also found to be negative by R-strip. This study demonstrated the influence of nonglucose-reducing substances to the Benedict's test, and ensured the specificity of this strip to be comparable to the commercial glucose oxidase strip.

Uric acid, creatinine, protein, drugs and many unknown factors in urine which effect the copper reduction test may interfere with the glucose oxidase strip test but others found that there was no affect, except for ascorbic acid[7,8,13,14]. Possible interference affecting the reaction are oxidizing agents which can cause a false positive reaction in the glucose oxidase test and low quantitative determination with the copper reduction test [15]. When half of R-strip was dipped into the urine, minichromatography developed. This approach was used in this present strip separating glucose from inhibitory substances, avoiding falsely negative results and making it more accurate than T-strips. It is not known whether all interference are separable from glucose.

It is clearly observable from this study that all four tests are useful for semi-quantitative determination of glucose in urine. All are definitely suitable for the diagnosis of diabetes mellitus and follow-up of the treatment or melliturla analysis, as long as their special qualities are realized. For evaluation inexperienced group tends to make a moderate reading if the color development is not extremely intense. Variation among an individual's judgement is responsible for a falsely low or falsely high reading with an unfamiliar strip like R-strip. However, R-strip is notable for excellent sensitivity especially at a lower glucose concentration. It is also specific and at least comparable to a commercial product.

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การทดสอบระดับน้ำตาลในปัสสาวะ : เปรียบเทียบแผ่นแถบสีที่ผลิตในห้องปฏิบัติการกับวิธีการทดสอบประจำ

*เรืองไรวรรณ คุณโฆษิต, วท.ม.**

ถึงแม้ว่าแผ่นแถบสีสำหรับตรวจระดับน้ำตาลกลูโคสในปัสสาวะ เป็นที่นิยมใช้แทนการตรวจโดยใช้หลอดทดลองมานานแล้ว แต่สำหรับประเทศไทยยังคงต้องนำเข้าจากต่างประเทศ เนื่องจากหลักการทดสอบเป็นปฏิกิริยาเอ็นซัยม์ สารเคมีในปฏิกิริยามีความไวต่อแสง และบริษัทผู้ผลิตไม่เปิดเผยสูตรและเคล็ดลับในการเตรียม การทดลองนี้ได้ผลิตแผ่นแถบสีโดยใช้กระดาษกรองพิเศษ ชุบเอ็นซัยม์สองชนิด และสารเคมีอีก 2 อย่าง ที่จะช่วยบ่งบอกโดยแปลงสีจากชมภูเป็นม่วงอ่อนหรือเข้มตามปริมาณของน้ำตาลกลูโคสที่พบในปัสสาวะ แผ่นแถบสีที่ผลิตได้นี้ตรวจสอบด้วยการใช้กลูโคสระดับมาตรฐานหลายระดับที่ตรวจพบบ่อย เติมลงในปัสสาวะเนกกาตีฟ โดยเปรียบเทียบกับแผ่นแถบสีสำหรับทดสอบระดับน้ำตาลกลูโคสในปัสสาวะของบริษัทและการตรวจหาระดับน้ำตาลในปัสสาวะโดยใช้หลอดทดลอง (Benedict's test) รวมทั้งวิธีการเดียวกัน ซึ่งใช้เม็ดยาจากบริษัทซึ่งใช้ประจำในห้องปฏิบัติการ ผลการศึกษาพบว่า แผ่นแถบสีมีความจำเพาะเท่าเทียมกับแผ่นแถบสีของบริษัทซึ่งใช้ประจำในห้องปฏิบัติการและมีความไวสูงกว่าวิธีอื่น การศึกษานี้คงเป็นแนวทางสำหรับการทดลองเพื่อเลือกซื้อแผ่นแถบสีหรือเพื่อพัฒนาการผลิตแผ่นแถบสีเพื่อตรวจหาระดับน้ำตาลในปัสสาวะ หรือเพื่อการทดสอบระดับสารเคมีอื่น ๆ ในปัสสาวะต่อไป

คำสำคัญ : แถบสีทดสอบกึ่งปริมาณ, น้ำตาลในปัสสาวะ, การตรวจด้วยเอ็นซัยม์กลูโคสอ็อกซิเดส

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