A Simple and Rapid Clinical Micro Method* for Determination of Urine Urea in 0.1 c.c. or Smaller Amounts.

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Formerly it was considered rather ridiculous to devise such a micro method for the urine as would require only 1 c.c. of it. Nowadays even a super-micro method of the urine will not be considered as useless, especially in the domain of pediatric biochemistry. In the present paper we desire to report of a super-micro method for the urine urea in $\frac{1}{100}$ c.c. of the material, not to say of 1 c.c.

Principle.

The principle of the method is very simple. Urea in $\frac{1}{100}$ c.c. of the urine is precipitated as dioxanthyl urea and the volume of the precipitate is volumetrically determined.

Amount to be Used.

1. Amount of urine required: $\frac{1}{10} - \frac{1}{100}$ c.c.

Usually when there is a sufficient amount of urine, it will be more convenient to use 0.1 or 1 c.c. of the urine. But when there is only 0.1 c.c. of the spare urine for the urea determination, then the amount may be diluted ten times with water and 0.1 c.c. of it corresponding $\frac{1}{100}$ c.c. of the undiluted urine, may be used.

2. Time required: About one hour for the whole technique up to the final determination.

* The 19th Report on Micro Method.

Reagents Required.

1. 20% sulfosalicylic acid solution (for the purpose of deproteinisation) (I).
2. X anthydrol solution (II).

Dissolve 1 gram. of xanthydrol ($\text{O} \langle \text{C}_6\text{H}_4 \rangle \text{CHOH}$) in 100 c. c. of pure glacial acetic acid and add to it 20 c. c. of pure methyl alcohol.

3. Standard urea solution. 0.01% aqueous urea solution (III).

Apparatus Required.

Besides the usual chemical apparatus, a few 1 c. c. graduated pipettes, 100 c. c. and 10 c. c. volumetric flasks and some calibrated test tubes of 9 millimeter diameter with a mark at 1 c. c. and 2.2 c. c. (Cf. Figure).

Technique.

1. In case of 1 c. c. of urine.

Put 1 c. c. urine into a test tube and add the some quantity of 20% sulfosalicylic acid (I) to it. If the latter is not sufficient, then add more of it.

Transfer this whole amount into a 10 c. c. volumetric flask and make up to the mark with water. Stir vigorously and filter.

Put 1 c. c. of the filtrate into one of the calibrated test tubes above described, and put in another 1 c. c. of 0.01% urea solution. Add 1.2 c. c. xanthydrol solution (II) to each test tube. Let them stand for 15 minutes and stir them vigorously. Then let them stand again for 25 minutes.

Compare the thickness of the floating precipitate of dixanthyl urea.

$$\frac{0.01 \times \text{Reading of unknown} (= \text{thickness of precipitate in unknown})}{5.25 (\text{Reading of standard solution} = \text{thickness of standard precipitate})} \times 100 = \text{percent of urea in urine}$$

2. In case of 0.1 c. c. of urine.

Put 0.1 c. c. of urine into a test tube and add 1 c. c. of 20% sulfosalicylic acid (I). Transfer into a 10 c. c. volumetric flask. Then procedure is the same as in the case of 1 c. c. of urine.

3. In case of 0.01 c.c. or $\frac{1}{100}$ c.c. of urine.

When the urine does not contain protein and there is only about one drop of the sample to spare, then an approximate amount of 0.01 c.c. may be taken and put into one of the calibrated test tubes. Then 1 c.c. of water is added. Then 20% sulfosalicylic acid is added and so on. In this case of course the result of determination is not so correct as in the case of the use of the larger amounts of the material.

0.04% 0.02% 0.01% Urea
standard solution.

Comparison of the Results between the Present Method and Yoshimatsu's¹⁾ Method applied to the Urine.

TABLE I.

The thickness of the precipitate of floating dioxanthylurea of the standard solutions in test tubes of 9 mm. in diameter.

Urea standard solution	Reading of the thickness of dioxanthyl urea			
	0.01% urea solution	0.02%	0.03%	0.04%
No. 1	5.0 mm.	9.5 mm.	14.5 mm.	20.0 mm.
No. 2	5.5 "	10.0 "	15.0 "	19.0 "
No. 3	5.0 "	11.0 "	14.0 "	19.5 "
No. 4	5.5 "	10.0 "	14.5 "	18.5 "
No. 5	6.0 "	11.0 "	15.0 "	19.0 "
No. 6	5.5 "	11.0 "	15.5 "	19.0 "
No. 7	5.5 "	10.5 "	14.5 "	20.0 "
No. 8	5.0 "	9.5 "	15.0 "	19.5 "
No. 9	6.0 "	10.0 "	14.5 "	20.5 "
No. 10	5.5 "	10.0 "	15.5 "	19.0 "
No. 11	5.0 "	11.0 "	14.5 "	19.0 "
No. 12	5.0 "	10.5 "	14.0 "	18.5 "
No. 13	5.0 "	9.5 "	14.5 "	20.0 "
No. 14	5.5 "	11.0 "	15.5 "	19.0 "
No. 15	5.0 "	9.5 "	14.5 "	20.5 "
No. 16	5.0 "	11.0 "	15.5 "	19.5 "
No. 17	5.0 "	10.0 "	14.5 "	18.5 "
No. 18	5.0 "	9.5 "	15.0 "	19.0 "
No. 19	5.5 "	10.0 "	14.5 "	18.5 "
No. 20	5.0 "	11.0 "	15.5 "	21.0 "
Average	5.25 "	10.25 "	14.8 "	19.4 "

1) Sh. Yoshimatsu, Tohoku J. Exp. Med., 1929, 13, 1.

Table I will show the result of the present determination method for theoretical solutions of different urea concentration (Cf. Table I).

Tables II and III will show the comparison of the results obtained by the present method and by Yoshimatsu's¹⁾ blood method applied to urine (Cf. Table II and Table III).

As is seen from these tables, the present method can be used for clinical purpose with a tolerable accuracy, especially as the technique is a very simple and rapid one.

TABLE II.

Comparison of figures (in urine) between Yoshimatsu's and the present method in human cases.

	Age (Years)	Sex	Percent of urea content in urine by Yoshimatsu's method	Percent of urea content in urine by Author's method	Differ- ence
No. 1	29	♂	1.49%	1.54%	+ 3.3%
No. 2	26	♂	1.62 „	1.6 „	- 1.2 „
No. 3	3	♀	0.95 „	0.83 „	- 12.6 „
No. 4	3½	♀	0.48 „	0.45 „	- 6.3 „
No. 5	15	♂	1.28 „	1.2 „	- 6.3 „
No. 6	2	♀	0.5 „	0.48 „	- 4 „
No. 7	52	♂	0.78 „	0.8 „	+ 2.5 „
No. 8	44	♂	0.38 „	0.4 „	+ 5.3 „
No. 9	22	♂	1.56 „	1.44 „	- 7.7 „
No. 10	29	♀	1.86 „	1.81 „	- 2.7 „
				Average	± 4 „

TABLE III.

Comparison of urea figures (in urine) between Yoshimatsu's and the present method in pathological conditions.

	Age (Years)	Sex	Diagnosis	Percent of urea content in urine by Yoshimatsu's method	Percent of urea content in urine by Author's method	Differ- ence
No. 1	32	♂	Nephritis chronica	0.85%	0.75%	- 1.2%
No. 2	38	♂	„	0.88 „	0.87 „	- 1.1 „
No. 3	3	♂	Nephritis	0.92 „	0.95 „	+ 3.3 „
No. 4	3	♂	„	0.97 „	0.95 „	- 2.2 „
No. 5	8	♀	Nephritis acuta	0.93 „	0.95 „	+ 2.6 „
No. 6	15	♂	„	1.15 „	1.12 „	- 2.6 „
No. 7	4	♂	„	2.54 „	2.66 „	+ 4.8 „
No. 8	22	♂	Uremia	0.57 „	0.47 „	- 17.5 „
No. 9	3	♀	Glomerulonephritis	0.92 „	0.9 „	- 2 „
No. 10	42	♂	Diabetes mellitus	1.45 „	1.42 „	- 2.1 „
				Average		± 5 „

Summary.

A clinical rapid micro or super-micro method for urea is described, which will enable one to determine urea in a minute amount of urine, such as 0.1 c. c. or smaller amounts.