

INFLUENCE OF STORAGE CONDITIONS AND PRESERVATIVES ON URINALYSIS

Authors:

Savale Apeksha¹, Sahu Shilpi², Kotecha Bansi^{3*}, Jadhav Priyanka⁴ and Samuel Stanley⁵

¹Post Graduate Student, Department of Pathology, Mahatma Gandhi Mission Medical College

²Professor and Head of Department of Pathology, Mahatma Gandhi Mission Medical College

³Post Graduate Student, Department of Pathology, Mahatma Gandhi Mission Medical College

⁴Associate Professor, Department of Pathology, Mahatma Gandhi Mission Medical College

⁵Post Graduate Student, Department of Pathology, Mahatma Gandhi Mission Medical College

***Corresponding Author:**

Dr. Bansi Kotecha

Department of Pathology,

Mahatma Gandhi Mission Medical College and Hospital,

Navi Mumbai-410209, Maharashtra, India.

Email: bansi.kotecha9@gmail.com

Article Received: 28-12-2022 Revised:13-01-2022 Accepted:28-01-2023

ABSTRACT:

Introduction: Urinalysis is very susceptible to pre-analytical issues, one of which arises due to improper or no preservation of urine samples before analysis. Thymol as a preservative because of its antimicrobial property has the advantage of preserving glucose and sediments well. The current study is to evaluate how different concentrations of preservative (thymol) used for storage of urine samples affected the outcome of urine analyses. **Aim and Objective:** To evaluate how refrigeration and use of preservative (thymol) for storage of urine samples affected the outcome of urine analysis. **Material and methods:** Spot urine samples were collected from 507 volunteers. Each fresh sample of urine was divided into three 10 ml aliquots, one without preservative, one with 1 drop of thymol and one with 2 drops of thymol. Each urine sample was analysed immediately after collection (0 hour) and after 24 hours. **Results:** Sugars were best preserved with the use of thymol and that specific gravity, urine protein, occult blood, BS/BP and urine ketones measured after 24 hours were similar to those at 0 hour when samples were stored at low temperature as well as with the use of thymol. **Conclusion:** Antibacterial preservatives like thymol can be recommended as preservatives for urine samples taking more than 2 hours. Ideally, the samples can be stored at 4 °C without any preservatives.

KEYWORDS: *Thymol, preservatives, urinalysis.*

INTRODUCTION:

Urinalysis is very susceptible to pre-analytical errors, one of which arises due to improper or no preservation of urine samples before analysis. And urinalysis being one of the most common tests performed in any laboratory, the need of the hour is to use Thymol as a preservative because of its antimicrobial property has the advantage of preserving glucose and sediments well. The current study is to evaluate how different concentrations of preservative (thymol) used for storage of urine samples affected the outcome of urine analyses.

AIM & OBJECTIVE:

To evaluate how refrigeration and use of preservative (thymol) for storage of urine samples affected the outcome of urine analyses.

MATERIAL AND METHODS:

This was a prospective study. Urine samples were obtained under strict aseptic precautions for urinalysis in sterile wide mouth containers. In our study, mid stream clean catch sample was used as the method of collection. Within 2 hours of collection, the urine samples were sent to central pathology laboratory. Each sample was divided into three groups(10 ml aliquots each): (A) - without preservative, stored at room temperature(22 °C), (B) - without preservative and stored at 4 – 8 °C (refrigeration), (C) - with preservative(thymol, crystal ~2 gm) at room temperature(20– 24°C). Urine analysis was performed by a trained technician and doctors at 0 hours and at 24 hours in all three groups. Urinalysis included both urine multistix dipstick test and microscopy. Patient's history was obtained from medical record department for clinical correlation. The results obtained from urinalysis performed at 24 hours were then compared with results obtained at 0 hours in each group. Data

was stored in MS Excel, and p value was calculated using SPSS test of significance. The probability of $p <$

0.05 was considered to be statistically significant.

RESULTS:

Samples were first analysed within 0 – 2 hours in all three groups. No significant changes in urinalysis of all three groups were found when urine was analysed at 0 hours. These were then compared results obtained after 24 hours.

Table 1: Comparing effects of refrigeration and thymol on biochemical findings of urinalysis

| PARAMETER | GROUP A 24 hours | GROUP B 24 hours | GROUP C 24 hours |
|------------------|---------------------|---------------------|---------------------|
| Specific gravity | × | √ | √ |
| pH | × | × | × |
| Sugar | × | × | √ |
| Protein | × | √ | √ |
| Occult blood | × | √ | √ |
| BS/BP | √ | √ | √ |
| Ketones | √ | √ | √ |

(BS/BP = Bile salts / Bile pigments, √ - no significant difference, × - significant difference).

Urinalysis done after 24 hours with no preservative showed significant changes in specific gravity, pH, urine sugars, urine protein and occult blood while results for bile salts/bile pigments (BS/BP) and urine ketones were consistent with findings at 0 hour. But on the other hand we found that specific gravity, urine protein, occult blood, BS/BP and urine ketones measured after 24 hours were similar to those at 0 hour when samples were stored at low temperature as well as with the use of thymol. Additionally we found that urine sugars were specifically preserved only with thymol.

Table 2: Comparing effects of refrigeration and thymol on microscopic findings of urinalysis

| PARAMETER | | GROUP A 24 hours | GROUP B 24 hours | GROUP C 24 hours |
|----------------|-----|---------------------|---------------------|---------------------|
| Microscopy | RBC | × | √ | √ |
| | PC | × | √ | √ |
| | EC | × | √ | √ |
| Crystals | | × | √ | √ |
| Other findings | | × | √ | √ |

(RBC = Red blood cells, PC = Pus cells, EC = Epithelial cells, √ - no significant difference, × - significant difference)

Microscopic findings of urinalysis 24 hours after refrigeration and on addition of thymol were similar to results of urinalysis done at 0 hours, showing the effectiveness of both methods of preservation.

Table 3: Statistical significance by paired t test

| PARAMETER | GROUP A 24 hours | GROUP B 24 hours | GROUP C 24 hours |
|-----------|---------------------|---------------------|---------------------|
| p value | < 0.05 | > 0.05 | > 0.05 |

After calculating p value, urinalysis results without the use of any preservative or refrigeration were found to be significantly different when compared with results reported at 0 hours. On the other hand, both refrigeration and use of thymol helped preserving urinalysis findings, which were statistically not significantly different from findings reported at 0 hours.

Table 4: % Concordance of urinalysis

| | |
|---|-------|
| Refrigeration(24 hours) and without preservative(0 hours) | 97.0% |
| Thymol(24 hours) and without preservative(0 hours) | 98.2% |

DISCUSSION:

In our study urine sugars were best preserved with the use of Thymol as preservative, which was in concordance with studies done by Wang X et al.¹ Our study also found no significant differences in urine protein, occult blood and microscopy findings with the use of preservative which is in concordance with studies done by Salazar-García S et al.², Nicar MJ et al.³, Feres MC et al.⁴ and Wang X et al.¹ Herrington W et al.⁵ in his study found storage of urine sample at 4°C helped preserve findings of albumin excretion in urine, which were in concordance with our study. No significant differences in urine protein, occult blood and microscopy findings when urine samples were refrigerated at 4 - 8 °C which was in concordance with the studies done by Wang X et al.¹ and Rebiero et al.⁶ Our study found that pH measured after 24 hours was significantly lower in all three groups which is in concordance with the studies done by Shafiee MA et al.⁷

CONCLUSION:

Thymol is the best preservative for testing urine sugars in urine taking more than 2 hours for analysis. Thymol, because of its antimicrobial property, preserves glucose and sediments very well and reduces bacterial infestation. Refrigeration is a good substitute to thymol to reduce bacterial growth and produce near accurate urinalysis results and these urine samples can be stored at 4 °C without any preservatives. This method is especially useful and extremely affordable in places where the sample load is very high and it is not possible to process every sample within 2 hours of collection.

In conclusion, even though thymol is an excellent preservative, refrigeration of urine samples is a highly feasible method of preservation, especially at a tertiary care hospital, for accurate urinalysis results.

FUNDING:

This research received no external funding.

ACKNOWLEDGMENTS:

This work was supported by the Mahatma Gandhi Mission Medical College and Hospital, Navi Mumbai.

CONFLICTS OF INTEREST:

The authors declare no conflict of interest.

REFERENCES:

1. Wang X, Gu H, Palma-Duran SA, Fierro A, Jasbi P, Shi X, Bresette W, Tasevska N. Influence of Storage Conditions and Preservatives on Metabolite Fingerprints in Urine. *Metabolites*. 2019 Sep 27;9(10):203. doi: 10.3390/metabo9100203. PMID: 31569767; PMCID: PMC6836253.
2. Salazar-García S, Lares-Villaseñor E, Bárcenas-Morales A, Vargas-Morales Juan M. Impact of chemical preservative in urine samples. *EJIFCC*. 2020 Mar 20;31(1):56-64. PMID: 32256289; PMCID: PMC7109501.
3. Nicar MJ, Hsu MC, Johnson T, Pak CY. The preservation of urine samples for determination of renal stone risk factors. *Lab Med*. 1987 Jun;18(6):382-4. doi: 10.1093/labmed/18.6.382. PMID: 11539109.
4. Feres MC, Bini R, De Martino MC, Biagini SP, de Sousa AL, Campana PG, Tufik S. Implications for the use of acid preservatives in 24-hour urine for measurements of high demand biochemical analytes in clinical laboratories. *ClinChimActa*. 2011 Nov 20;412(23-24):2322-5. doi: 10.1016/j.cca.2011.08.033. Epub 2011 Sep 2. PMID: 21910978.
5. Herrington W, Illingworth N, Staplin N, Kumar A, Storey B, Hrusecka R, Judge P, Mahmood M, Parish S, Landray M, Haynes R, Baigent C, Hill M, Clark S. Effect of Processing Delay and Storage Conditions on Urine Albumin-to-Creatinine Ratio. *Clin J Am Soc Nephrol*. 2016 Oct 7;11(10):1794-1801. doi: 10.2215/CJN.13341215. Epub 2016 Sep 21. PMID: 27654930; PMCID: PMC5053802.
6. Ribeiro, K. C. B., Serabion, B. R. L., Nolasco, E. L., Vanelli, C. P., Mesquita, H. L. de, & Corrêa, J. O. do A. (2013). *Urine storage under refrigeration preserves the sample in chemical, cellularity and bacteriuria analysis of ACS. Jornal Brasileiro de Patologia e Medicina Laboratorial*, 49(6), 415–422. doi:10.1590/s1676-24442013000600006.
7. Shafiee, MA, Shaker, P., Hosseini, S. F., Alavinia, M., Aarabi, M., Rezaee, A. J., & Halperin, M. L. (2021). Are individual analyses of multiple short urine collections throughout the 24 hours superior to a standard 24-hour urine collection in precipitation risk assessment of healthy subjects? *Nephrology*, 26(3), 234–238. doi:10.1111/nep.13842