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# A STUDY OF THE STABILITY OF BIOCHEMICAL ANALYTES IN SERUM OF HEALTHY POPULATION

**Biochemistry**

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## ABSTRACT

**Background:** This is a lab based study and the study was carried out to study the stability of routine biochemical analytes in serum of healthy population. Errors in the lab at preanalytical phase contribute to a significant number. This type of error can be minimized by subjecting sample to a proper storage and transport. Any variation in the storage condition may lead to erroneous result. **Aim:** To study serum stability of routine biochemical parameters in a healthy population **Methods:** This is an experimental study (laboratory based study). 51 healthy personnel from the tertiary care centre of Defence establishment in Pune during the study period (Aug 2022 to Sep 2022) were included in the study. Random sampling of the subjects was done from defined study population. 5 ml of venous blood was collected in yellow topped vacutainer and was allowed to clot for 1 hour. Thereafter, serum was separated by centrifuging the sample at 3000 rpm for 3 minutes. Total of 5 aliquotes of serum was made. 2 aliquotes were kept in refrigerator at 4 degree Celsius and 3 aliquotes were kept at room temperature. First analysis of all the analytes including urea, creatinine, sodium, potassium, lactate dehydrogenase (LDH), Calcium, phosphorous, uric acid and total cholesterol were made within 1 hour of serum separation. Second analysis of all the analytes were made after 5 hours of serum separation from both the aliquots kept at room temperature and in refrigerator similarly third aliquots from refrigerator and room temperature were analyzed after 24 hours of serum separation. Statistical analysis was performed using GraPad Prism Version 9 software. One way ANOVA was applied for multiple comparisons. P value less than 0.05 was considered as statistically significant. . **Results:** Urea, creatinine, LDH, uric acid and total cholesterol did not show any statistically significant variation in the values when stored at room temperature up to 24 hours or is refrigerated at 4 degree Celsius up to 24 hours. Total protein values shows that there is significant differences in sample stored at 24 hours at 4 degree Celsius when compared to sample analysed within 1 hour of serum separation (baseline). Potassium showed significant changes for value of sample stored at 24 hour at room temperature with that of baseline value. Sodium showed significant changes for value of sample stored at 24 hour at room temperature with that of baseline value. Calcium values shows that there is significant changes for sample stored at 24 hours at 4 degree Celsius with that sample analysed within 1 hour of serum separation. **Conclusion:** It is observed in present study that urea, creatinine, LDH, uric acid and total cholesterol can be analysed without any changes in the quality of reports when stored at room temperature or is refrigerated at 4 degree Celsius. Values of total protein, potassium, sodium and calcium showed statistically significant changes though clinically it is not significant, Hence, while analyzing and interpreting results for these analytes, effect of storage conditions should be kept in mind

## KEYWORDS

Serum, stability, temperatures

## INTRODUCTION:

All the investigations in the lab passes through three different types of errors namely preanalytical, analytical and postanalytical phase [1]. Errors in preanalytical phase are those which comes before the analysis like errors during sample collection, sample transportation, sample storage etc. Errors in analytical phase occurs due to instrument malfunction, errors in quality controls etc. Postanalytical errors occurs due to wrong result entry, interpretation errors etc. These errors can be minimized to a great extent by certain precautions taken during that period. Rejection of most of the samples occurs during the preanalytical phase only due to any errors mentioned above. Errors in the lab at preanalytical phase contribute to a significant number.

This type of error can be minimized by subjecting sample to a proper storage and transport. All the analytes in the serum are subjected to degradation and stability changes. It is pertinent to understand that these biochemical analytes in the serum are stable up to a particular temperature only [2]. Any variation in the storage condition may lead to erroneous result. In the era of accreditation, patient safety priority and quality control, the value of these lab results are very precious. Stability of any analytes depends on many factors like patient preparation, sample collection, sample storage, sample transportation and sample analysis. Any deviation from the laid down guidelines at any stage of sample analysis may results in erroneous lab results [3].

Most of the biochemical analytes estimated in the lab undergoes many changes which might leads to degradation of the analytes and causes false results [4]. It is must to know the stability of common biochemical analytes at different conditions so that the results can be interpreted with caution and we will be able to help the clinicians and finally to the patients. Collection and handling of sample is prerequisite for stability limit of an analyte [5] There are procedures to transport samples from clinicians to the laboratories[6].

Previous study showed that most of the analytes investigated remained stable up to 24h under all storage conditions [3]. However, some analytes were highly affected due to temperature or delay in transport [7]. The collected and transported blood samples are exposed to a many extra-analytical factors before analysis[8].

Present study was performed keeping in mind the sample storage at different time and storage temperatures so that it will be easy to know the integrity of the analytes at different storage time. However, study had showed analyte integrity at six different storage conditions for analytes[9.

Study has shown that the value of some of the analytes like glucose, total bilirubin and albumin had significant change when baseline value (analysed within 2 hours of sample collection) compared with the

value when sample stored at 2 to 8 degree Celsius for 24 hours [10]. MarjaniA et al studied to determine the effects of storage time and temperature on the laboratory results of 10 analytes in sera from apparently healthy adult male and he recommended that storage at definite temperature is must after the sample is collected[11].

Quality of lab results will be improved if various pre analytical factors like errors in sample collection, sample transportation and sample storage are considered before the lab results [4]. Handling and storage of the samples is good practices to provide quality lab results [12,13]. Proper handling of the samples to avoid hemolysis, transporting the samples at correct temperatures and storage of samples at appropriate temperatures before analysis are some of the good lab practices.

Correct preanalytical procedure is very important to get adequate sample and good quality lab results and hence patient safety [14]. Recognition of pre analytical factors is very important for the labs and should be recognised [15].There should be good practice in lab to follow the safe lab practices[16].

Study by Taylor et al showed that the minimal effects on sample stability were noted for the majority of analytes using the storage conditions tested in the study [17]. Study by Tanner et al showed that many analytes like sodium, total protein, albumin, bilirubin, alanine transferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transferase were unaffected by a doing late centrifugation at different temperatures, however, some important analytes like potassium, glucose, phosphate, creatinine, urea, ferritin, iron were significantly affected [18].

The study was conducted keeping in mind the following aim and objectives:
**Aim:** To study serum stability of routine biochemical parameters in a healthy population

**Objectives:**
1) To analyze different biochemical lab parameters ( total protein, urea, creatinine, potassium, sodium, lactate dehydrogenase, calcium, phosphorous, uric acid, total cholesterol ) in serum stored at different storage temperatures.
2) To analyze different biochemical parameters (mentioned above) in serum stored at different time duration period.
3) To compare all biochemical lab parameters (mentioned above) with the baseline value (after 1 hour of serum separation).

**METHODS:**
**Type of study:** Prospective study
**Study design:** Experimental study
**Study setting:** Laboratory based study.
**Sample size:** Sample size was calculated statistically considering $\alpha = 0.05$, $1-\beta = 0.80$, Mean and Standard deviation (SD) from previous studies for each analytes value before and after storage and highest sample size for the analyte is 51 [8,10,18].

**Study population:** 51 healthy personnel from the institute during the study period (Aug 2022 to Sep 2022) were included in the study. Study was done in a tertiary care centre of Defence establishment in Pune.

**Sampling**: Random sampling of the subjects were selected from defined study population.

**Selection criteria:** Healthy subjects were included in the study.
**Exclusion criteria**: Subjects with proven disability like diabetes mellitus, hypertension, Coronary artery disease, hypothyroidism and subjects on medications for any illnesses were excluded from the study.

**Study protocol:** Ethical clearance was obtained from institutional ethical committee with IEC no IEC S No: IEC/2022/86 dated 09 May 2022. All guidelines as per declaration of Helsinki and good clinical practice guidelines were followed. 51 healthy young volunteered healthy subjects were recruited in the study after taking written informed consent from each of them. 5 ml of venous blood was collected in yellow topped vacutainer and was allowed to clot for 1 hour. Thereafter, serum was separated by centrifuging the sample at 3000 rpm for 3 minutes and 5 aliquotes of serum were made. 2 aliquotes were kept in refrigerator at 4 degree Celsius and 3 aliquotes were kept at room temperature( 25 degree Celsius). First analysis of all the analytes total protein, urea, creatinine, sodium, potassium, lactate dehydrogenase (LDH), Calcium, phosphorous, uric acid and total cholesterol were made within 1 hour of serum separation using fully automated Biochemistry analyzer (Siemens Dimension EXL 200). Second analysis of all the analytes were made after 5 hours of serum separation from both the aliquots kept at room temperature and in refrigerator at 4 degree celsius, Similarly third aliquots from refrigerator and room temperature were analyzed after 24 hours of serum separation.

All the analytes were analyzed after the 2 levels of quality control values were run and found within ±2SD at fully automated biochemistry analyzer manufactured by Siemens healthcare Pvt Ltd with Model of Dimension EXL-200 The principles of each analytes are as follows:

**Serum urea estimation**
Urea Nitrogen

**Principle:**
Urease specifically hydrolyses urea to form ammonia and carbon dioxide.The ammonia is used by the enzyme glutamate dehydrogenase (GLDH) to reductively aminate alpha-ketoglutarate ,with simultaneous oxidation of reduced nicotinamide dinucleotide (NADH). The change in absorbance at 340nm due to disappearance of NADH is directly proportional to the BUN concentration inthe sample and is measured using a bichromatic (340,383nm) rate technique.

**Serum creatinine estimation**
Modified kinetic Jaffe's technique

**Principle:**
The method uses a modified kinetic Jaffe technique. In the presence of a strong base such as NaOH, picrate reacts with creatinine to form a red chromophore.The rate of increasing absorbance at 510 nm due to formation of this chromophore isdirectly proportional to the creatinine concentration in the sample and is measured using a bichromatic (510,600nm) rate technique. Bilirubin is oxidised by potassium ferrocyanide to prevent interference.

**Serum uric acid estimation**
**Principle:**
Uric acid which absorb light at 293 nm is converted by Uricase to allantoin, which isnon absorbing at 293 nm. The change in absorbance at 293 nm due to disappearance even of uric acid is directly proportional to the concentration of Uric acid in the sample and is measured using a bichromatic (293, 700nm) endpoint technique.

**Serum phosphorous estimation**
**Principle:**
Inorganic phosphate reacts with ammonium molybdate in the presence of sulfuric acid to form a phosphomolybdate complex which is measured at 340 nm and blanked at 700nm.

**Serum total Cholesterol estimation**
**Principle:**
Cholesterol esterase (CE) catalyses the hydrolysis of cholesterol esters to produce free cholesterol which , along with pre-existing free cholesterol, is oxidised in a reaction catalysedby cholesterol oxidase (CO) to form cholest-4-ene-3-one and a hydrogen peroxide. In the presence of horseradish peroxide (HPO) , the hydrogen peroxide thus formed is used to oxidiseN,N,diethylalanine-HCL/4-aminoantipyrine(DEA-HCL/AAP) to produce a chromophore that absorbs at 549 nm. The absorbance due to oxidized DEA-HCL/AAP is directly proportional to the total cholesterol concentration and is measured using a polychromatic (452,540,700 nm) endpoint technique.

**Serum calcium estimation**
**Principle:**
Calcium reacts with O-CresolphthaleinComplexone (OCPC) to form a purple complex. The amount of complex thus formed is proportional to the calcium concentration and is measured using a bichromatic(577, 540 nm) endpoint technique. Magnesium ions, which also form a colored complex with OCPC, are removed from the reaction by complexation with 8-quinolinol.

**Serum sodium estimation**

**Principle:**
There are four electrodes used to measure electrolytes on the Dimension® system. Three of these electrodes are incorporated into the QuikLYTE® Integrated Multisensor and are ion selective for sodium, potassium and chloride. A reference electrode is also incorporated in the multisensor. After a diluted sample is positioned in the sensor, Na+, K+ and Cl- ions establish an equilibrium with the electrode surface. A potential is generated proportional to the logarithm of the analyte activity in the sample. The electrical potential generated on a sample is compared to the electrical potential generated on a standard solution, and the concentration of the desired ions is calculated by use of the Nernst equation.1,2,3

**Serum potassium estimation**
**Principle:**
There are four electrodes used to measure electrolytes on the Dimension® system. Three of these electrodes are incorporated into the QuikLYTE® Integrated Multisensor and are ion selective for sodium, potassium and chloride. A reference electrode is also incorporated in the multisensor. After a diluted sample is positioned in the sensor, Na+, K+ and Cl- ions establish an equilibrium with the electrode surface. A potential is generated proportional to the logarithm of the analyte activity in the sample. The electrical potential generated on a sample is compared to the electrical potential generated on a standard solution, and the concentration of the desired ions is calculated by use of the Nernst equation.1,2,3

**Serum total protein estimation**
**Principle:**
Cupric ions (Cu++) reacts with the peptide linkages of protein in a basic solution . The blue copper (ll) protein complex thus formed is proportional to the total protein concentration in the sample and is measured using a bichromatic (540,700nm) endpoint technique.

**Serum lactate Dehydrogenase estimation**
**Principle:**
The LDI method uses a substrate L-Lactate buffered at a ph of 9.4. Lactate dehydrogenase oxidises the substrate in the presence of NAD+ to yield pyruvate and NADH which absorbs at 340 nm . Lactate dehydrogenase activity concentration is measured as a rate reaction at 340/700nm, proportional to the amount of lactate dehydrogenase in the sample.

**Statistical Analysis:**
Statistical analysis was performed using GraPad Prism Version 9 software. One way ANOVA was applied for comparison of values. All the values (table 1) are expressed in mean±SD with adjusted p values. P value of <0.05 were considered significant.

**RESULTS:**
Statistical analysis was performed using GraPad Prism Version 9 software. One way ANOVA was applied for comparison of values. All the values ( 5 hr room temp, 5 hr 4 degree Celsius, 24 hr room temp & 24 hr 4 degree Celsius) were compared with the baseline values (analyzed within 1 hour of serum separation). Table 1 shows the mean, SD and p value of all the analytes. Multiple comparison for total protein values shows that there is significant differences in sample stored at 24 hours at 4 degree Celsius when compared to sample analysed within 1 hour of serum separation (baseline) with a p value of < 0.001. All other values stored at different storage conditions did not show any significance difference compared to the baseline values. Multiple comparison of potassium showed significant changes for value of sample stored at 24 hour at room temperature with that of baseline value with a p value of < 0.001 and rest other values did not show any significant changes. Multiple comparison of sodium showed significant changes for value of sample stored at 24 hour at room temperature with that of baseline value with a p value of < 0.001 and rest other values did not show any significant changes. Multiple comparison for calcium values shows that there is significant changes for sample stored at 24 hours at room temperature and at 4 degree Celsius with that sample analysed within 1 hour of serum separation with a p value of < 0.001. Phosphorous shows there is significant changes for value of sample stored at for 24 hours at room temperature. All other values stored at different storage conditions did not show any significance compared to the baseline values. Multiple comparison of urea, creatinine, LDH, uric acid and total cholesterol did not show any significant changes. Scatter plot diagrams of all the analytes demonstrating changes with time is shown (Fig 1 to 10).

| Analytes | | 1 Hr (Room Temp) | 5 Hr (Room Temp) | 5 Hr( 4 degree C) | 24 Hr (Room Tem) | 24 Hr (4 degreeC) |
|---|---|---|---|---|---|---|
| Total Protein | Mean±SD | 7.839±0.2911 | 7.859±0.3028 | 7.836±0.2854 | 7.895±0.3224 | 8.089±0.3468 |
| | Adjusted p value | - | 0.99 | 0.99 | 0.76 | <0.001 |
| Urea | Mean±SD | 11.25±2.452 | 11.17±2.703 | 11.3±2.460 | 11.38±2.548 | 11.48±2.546 |
| | Adjusted p value | - | 0.99 | 0.99 | 0.99 | 0.97 |
| Creatinine | Mean±SD | 1.041±0.1426 | 1.05±0.1492 | 1.012±0.1587 | 0.991±0.1387 | 0.9752±0.1563 |
| | Adjusted p value | - | 0.99 | 0.72 | 0.26 | 0.09 |
| Potassium | Mean±SD | 4.471±0.3274 | 4.475±0.3326 | 4.445±0.3334 | 4.822±0.3774 | 4.535±0.3465 |
| | Adjusted p value | - | 0.99 | 0.99 | <0.001 | 0.75 |
| Sodium | Mean±SD | 145.4±2.006 | 146.1±2.437 | 145.4±2.4 | 157.5±7.643 | 148.3±3.425 |
| | Adjusted p value | - | 0.77 | 0.99 | <0.001 | 0.001 |
| LDH | Mean±SD | 198.5±39.49 | 194.6±39.16 | 193.7±39.18 | 199.3±41.38 | 187.8±38.05 |
| | Adjusted p value | - | 0.96 | 0.93 | 0.99 | 0.44 |
| Calcium | Mean±SD | 9.311±0.2948 | 9.281±0.2683 | 9.257±0.2654 | 9.452±0.3225 | 9.673±0.3291 |
| | Adjusted p value | - | 0.96 | 0.77 | 0.05 | <0.001 |
| Phosporous | Mean±SD | 3.449±0.6039 | 3.608±0.6264 | 3.471±0.6059 | 3.526±0.6082 | 3.548±0.6236 |
| | Adjusted p value | - | 0.96 | 0.77 | 0.05 | <0.001 |
| Uric Acid | Mean±SD | 6.109±1.275 | 6.247±1.248 | 6.211±1.263 | 6.293±1.275 | 6.411±1.3 |
| | Adjusted p value | - | 0.95 | 0.98 | 0.87 | 0.56 |
| Total cholesterol | Mean±SD | 171.7±37.37 | 172±37.37 | 171.3±37.83 | 172.7±37.83 | 176±37.33 |
| | Adjusted p value | - | 0.99 | 0.99 | 0.99 | 0.94 |

[Table 1]: Mean, standard deviation (SD) and p value of all the analytes analysed at different time durations
Units of measurements: Total protein (g/dL), Urea (mg/dL), Creatinine (mg/dL), Potassium (mmol/L), Sodium(mmol/L),LDH (IU/L),Calcium(mg/dL),Phosphorous(mg/dL),Uric acid(mg/dL),Total Cholesterol(mg/dL)

**Fig 1 to 10 X-axis showing time durations and Y-axis showing the results**

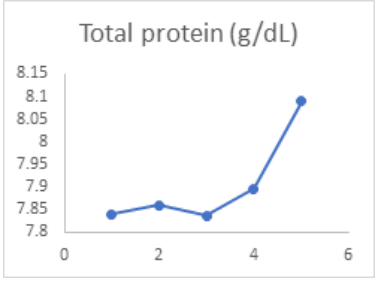


**Fig 1**

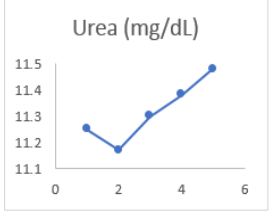


**Fig 2**

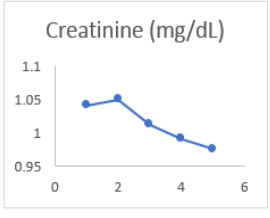


**Fig 3**

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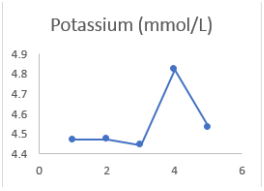


Fig 4

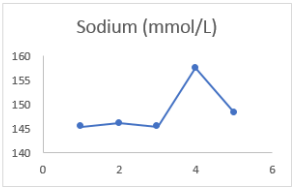


Fig 5

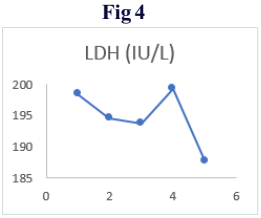


Fig 6

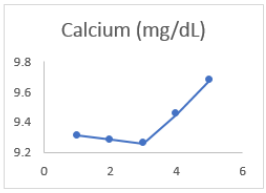


Fig 7

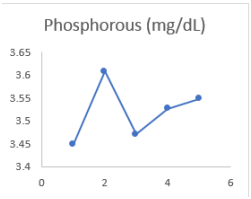


Fig 8

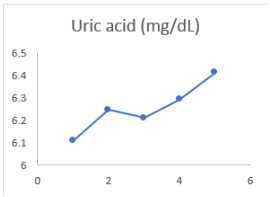


Fig 9

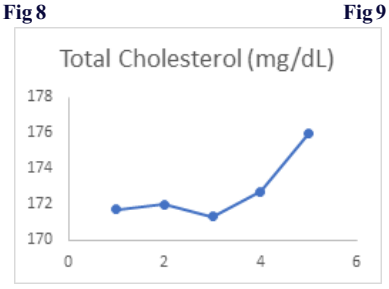


Fig 10

## DISCUSSION:

It is observed in our study that the values of total protein, potassium, sodium, calcium and phosphorous showed statistically significant changes whereas values of urea, creatinine, LDH, uric acid and total cholesterol did not show any significant changes when the serum was stored at different storage conditions up to 24 hours.

Value of total protein in present study is significantly high statistically in sample preserved at 4 degree Celsius compared to the baseline sample though clinically this value is insignificant. This may be explained by the fact that sample size in the study was small. Study done by Cuhadar et al where they stored the sample at 4 degree and 24 degree Celsius for 6,12,18,24,30,36 hrs and found that total protein remained stable throughout the storage conditions [8].

Study done by Kughapriya et al where they stored the serum at four different storage conditions first baseline value within two hours, other three stored at room temperature for four hours, 2-8 degree Celsius for four hours and third 2-8 degree Celsius for 24 hours [10]. They concluded that baseline serum value of total protein showed significant changes when baseline values were compared with the values analyzed after four hours at room temperature but when the baseline values were compared with the value of 24 hour results it did not show any significant changes. Values of urea in the study was not showing any significant changes at different storage and time duration periods. Same study showed significant changes for urea at baseline value compared to sample stored for four hours at 2-8 degree Celsius.

Study done by Marjani A et alshowed no effect of urea value in sample stored at 4±1 degree Celsius and 23±1 degree Celsius for as long as 72 hours [11]. Values of creatinine in the study was not showing any significant changes at different storage and time duration periods. Study done by Tanner M et al showed that creatinine was increased in sample stored at 25 degree celsius and 35 degree Celsius for 24 hours [18]. However in the study creatinine was initially increased at 5 degree Celsius at room temperature but it decreases at samples stored at room temperature for 24 hours and also at 4 degree Celsius at 24 hours which is clinically insignificant. Values of potassium in the study was not showing any significance however value of potassium at baseline level was significantly different from value at 24 hours sample kept at room temperature which may be due to the evaporation of sample and its contact with air.

Study done by Christiane Oddoze et al showed that potassium was significantly affected by storing sample at 24 hours [7]. Same study showed that lactate dehydrogenase were significantly affected either by delay, tube type or temperature up to 24 hour storage. Values of calcium in the study showed no significance except the value at 24 hour duration stored at room temperature and at 4 degree Celsius when compared to the baseline values. Though there was a significance but clinically it was not significant, which may be explained by smaller sample size.

Marjani A et alstudied the effect of storage at room temperature (23±1 degree Celsius) and refrigeration (4±1 degree Celsius) for 0,1,2,3,4,5,6,7,8,24,48 and 72 hours on 10 sera analytes were investigated and they found that values of calcium was not affected at any storage conditions. Values of phosphorous in the study showed no significance except the value at 24 hour duration stored at 4 degree Celsius when compared to the baseline values. The value of potassium were affected by storage at 4±1 degree Celsius and 23±1 degree Celsius for 48 hour and 24 hour respectively. Values of uric acid and total Cholesterol in the study did not show any significance at any time duration [11].

Changes in the values as mentioned above can be avoided by proper storage of the samples as per laid down guidelines by NABL 112 updated issue of ISO 15189:2012 or formulating standard operating procedures based on laid down guidelines.

## CONCLUSION

It is observed in present study that values of urea, creatinine, LDH, uric acid and total cholesterol did not show any significant changes when the serum was stored at different storage conditions up to 24 hours and hence these analytes can be analysed without any compromise in the quality of reports when stored at room temperature or is refrigerated at 4 degree Celsius. Values of total protein, potassium, sodium, calcium and phosphorous showed statistically significant changes though clinically it is not significant, Hence, while analyzing and interpreting results for these analytes,effect of storage conditions should be kept in mind

### Limitations

Present study is being conducted for 10 analytes only.

### Future perspectives

Further study can be conducted taking into consideration maximum biochemical analytes including hormones, tumour markers and other analytes.

### Acknowledgments

Article is based on ICMR-STS project

### Conflict of interests

None

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