

Webtable 3: Mean percentage change in urinary creatinine concentration, albumin concentration and albumin:creatinine ratio in urine with preservative following a delay in processing, by baseline level of albuminuria and storage temperature

	Mean percentage change (95% CI)		Test for equality of means by temperature
	Macroalbuminuria	Normo- or microalbuminuria	
Creatinine			
4°C, 1 day	-0.4% (-0.7 to -0.1%)	0.1% (-0.4 to 0.5%)	0.46
4°C, 2 days	-0.0% (-0.4 to 0.4%)	0.2% (-0.2 to 0.7%)	
4°C, 4 days	-0.2% (-0.6 to 0.2%)	0.1% (-0.3 to 0.5%)	
4°C, 7 days	0.8% (0.2 to 1.3%)	0.5% (0.1 to 0.9%)	
18°C, 1 day	-0.4% (-0.8 to 0.1%)	0.1% (-0.4 to 0.5%)	0.97
18°C, 2 days	-0.2% (-0.5 to 0.2%)	0.1% (-0.3 to 0.5%)	
18°C, 4 days	-0.1% (-0.8 to 0.7%)	-0.1% (-0.7 to 0.5%)	
18°C, 7 days	0.7% (-0.7 to 2.0%)	0.0% (-0.7 to 0.7%)	
30°C, 1 day	-0.6% (-1.0 to -0.2%)	-0.5% (-1.0 to -0.0%)	0.09
30°C, 2 days	-0.5% (-1.0 to 0.1%)	-0.8% (-1.2 to -0.3%)	
30°C, 4 days	-0.3% (-2.0 to 1.5%)	-1.8% (-2.5 to -1.0%)	
30°C, 7 days	-1.3% (-3.1 to 0.4%)	-2.9% (-4.0 to -1.7%)	
Albumin			
4°C, 1 day	-3.5% (-6.4 to -0.6%)	1.1% (-0.6 to 2.7%)	0.01
4°C, 2 days	-4.3% (-6.4 to -2.2%)	-0.5% (-2.8 to 1.7%)	
4°C, 4 days	-2.7% (-5.5 to 0.1%)	0.8% (-0.8 to 2.3%)	
4°C, 7 days	-0.2% (-2.1 to 1.8%)	0.6% (-2.0 to 3.2%)	
18°C, 1 day	-2.5% (-4.8 to -0.2%)	1.8% (-0.9 to 4.5%)	0.04
18°C, 2 days	-2.7% (-4.9 to -0.5%)	0.8% (-1.0 to 2.6%)	
18°C, 4 days	-1.1% (-4.3 to 2.1%)	1.6% (-0.8 to 4.1%)	
18°C, 7 days	2.1% (-0.3 to 4.4%)	2.1% (-0.2 to 4.4%)	
30°C, 1 day	-0.3% (-2.5 to 1.9%)	1.3% (-0.8 to 3.4%)	0.11
30°C, 2 days	-0.8% (-3.1 to 1.5%)	2.5% (0.2 to 4.7%)	
30°C, 4 days	0.2% (-4.4 to 4.7%)	4.0% (1.1 to 6.8%)	
30°C, 7 days	4.2% (0.6 to 7.8%)	4.7% (1.4 to 8.0%)	
Albumin:creatinine ratio			
4°C, 1 day	-3.1% (-6.1 to -0.1%)	0.9% (-0.8 to 2.6%)	0.02
4°C, 2 days	-4.2% (-6.4 to -2.1%)	-0.8% (-3.2 to 1.5%)	
4°C, 4 days	-2.5% (-5.3 to 0.3%)	0.6% (-1.0 to 2.2%)	
4°C, 7 days	-0.9% (-3.0 to 1.2%)	0.1% (-2.5 to 2.7%)	
18°C, 1 day	-2.2% (-4.5 to 0.2%)	1.8% (-1.0 to 4.5%)	0.04
18°C, 2 days	-2.5% (-4.7 to -0.4%)	0.7% (-1.2 to 2.5%)	
18°C, 4 days	-1.0% (-4.2 to 2.2%)	1.7% (-0.8 to 4.2%)	
18°C, 7 days	1.4% (-0.9 to 3.7%)	2.1% (-0.2 to 4.3%)	
30°C, 1 day	0.3% (-2.0 to 2.5%)	1.7% (-0.3 to 3.8%)	0.03
30°C, 2 days	-0.3% (-2.7 to 2.0%)	3.2% (0.9 to 5.5%)	
30°C, 4 days	0.4% (-4.3 to 5.1%)	5.7% (3.2 to 8.3%)	
30°C, 7 days	5.6% (2.6 to 8.6%)	7.8% (4.4 to 11.1%)	

Webtable 1: Mean percentage change in urinary creatinine concentration, albumin concentration and albumin:creatinine ratio after freeze-thaw cycles, by preservative use

	Mean percentage change (95% CI)		Test for equality of means
	Preservative	No preservative	
Creatinine			
One freeze-thaw	-0.1% (-0.4 to 0.2%)	-0.4% (-0.9 to 0.1%)	0.36
Two freeze-thaws	-0.0% (-0.3 to 0.3%)	0.0% (-0.2 to 0.3%)	0.74
Three freeze-thaws	0.1% (-0.2 to 0.4%)	0.3% (-0.0 to 0.6%)	0.47
Albumin			
One freeze-thaw	-0.8% (-2.3 to 0.7%)	0.3% (-2.0 to 2.6%)	0.39
Two freeze-thaws	-1.1% (-3.3 to 1.0%)	1.3% (-1.5 to 4.0%)	0.06
Three freeze-thaws	-0.7% (-3.0 to 1.7%)	2.8% (-0.3 to 6.0%)	0.03
Albumin:creatinine ratio			
One freeze-thaw	-0.7% (-2.2 to 0.9%)	0.6% (-1.6 to 2.8%)	0.28
Two freeze-thaws	-1.2% (-3.4 to 0.9%)	1.1% (-1.6 to 3.9%)	0.06
Three freeze-thaws	-1.0% (-3.3 to 1.4%)	2.3% (-0.7 to 5.4%)	0.04

Webtable 4: Mean percentage change in urinary creatinine concentration, albumin concentration and albumin:creatinine ratio in urine with preservative after freeze-thaw cycles, by baseline level of albuminuria

	Mean percentage change (95% CI)		Test for equality of means
	Macroalbuminuria	Normo- or microalbuminuria	
Creatinine			
One freeze-thaw	-0.3% (-0.7 to 0.2%)	-0.0% (-0.5 to 0.4%)	0.46
Two freeze-thaws	-0.2% (-0.6 to 0.3%)	0.0% (-0.4 to 0.4%)	0.59
Three freeze-thaws	0.1% (-0.3 to 0.5%)	0.1% (-0.3 to 0.5%)	0.95
Albumin			
One freeze-thaw	-3.6% (-6.1 to -1.1%)	0.5% (-1.3 to 2.3%)	0.01
Two freeze-thaws	-4.5% (-7.6 to -1.3%)	0.2% (-2.5 to 2.9%)	0.03
Three freeze-thaws	-3.5% (-6.4 to -0.5%)	0.2% (-2.9 to 3.3%)	0.09
Albumin:creatinine ratio			
One freeze-thaw	-3.3% (-5.8 to -0.8%)	0.6% (-1.3 to 2.4%)	0.02
Two freeze-thaws	-4.3% (-7.3 to -1.3%)	0.1% (-2.6 to 2.9%)	0.03
Three freeze-thaws	-3.6% (-6.5 to -0.6%)	0.1% (-3.1 to 3.2%)	0.10

Webtable 2: Mean percentage change in urinary creatinine concentration, albumin concentration and albumin:creatinine ratio after 1 and 6 month storage, by preservative use and storage temperature

	Mean percentage change (95% CI)		Test for equality of means
	Preservative	No preservative	
Creatinine			
1 month, -20°C	-0.1% (-0.5 to 0.3%)	0.6% (-0.3 to 1.4%)	0.14
1 month, -40°C	0.1% (-0.5 to 0.7%)	0.4% (-0.5 to 1.3%)	0.58
1 month, -80°C	0.1% (-0.7 to 1.0%)	0.5% (-0.6 to 1.5%)	0.62
6 months, -20°C	1.7% (1.3 to 2.1%)	3.1% (2.0 to 4.3%)	0.01
6 months, -40°C	0.1% (-0.3 to 0.5%)	1.1% (0.2 to 2.0%)	0.03
6 months, -80°C	0.1% (-0.3 to 0.6%)	0.7% (-0.2 to 1.6%)	0.27
Albumin			
1 month, -20°C	-9.0% (-12.2 to -5.8%)	-3.8% (-8.3 to 0.6%)	0.04
1 month, -40°C	-1.1% (-3.1 to 0.9%)	-2.0% (-6.5 to 2.5%)	0.70
1 month, -80°C	-2.2% (-5.5 to 1.2%)	-1.2% (-6.1 to 3.6%)	0.73
6 months, -20°C	-22.6% (-29.3 to -15.9%)	(-26.2 to -12.1%)	0.37
6 months, -40°C	-2.1% (-4.1 to -0.1%)	-2.2% (-6.5 to 2.0%)	0.96
6 months, -80°C	-0.1% (-2.5 to 2.3%)	1.1% (-3.9 to 6.1%)	0.67
Albumin:creatinine ratio			
1 month, -20°C	-8.9% (-12.1 to -5.7%)	-4.4% (-9.4 to 0.7%)	0.11
1 month, -40°C	-1.3% (-3.4 to 0.7%)	-2.4% (-7.6 to 2.8%)	0.68
1 month, -80°C	-2.8% (-6.3 to 0.7%)	-1.8% (-7.2 to 3.7%)	0.73
6 months, -20°C	-23.9% (-30.5 to -17.4%)	(-28.7 to -14.5%)	0.54
6 months, -40°C	-2.3% (-4.4 to -0.2%)	-3.3% (-8.2 to 1.5%)	0.68
6 months, -80°C	-0.4% (-3.0 to 2.2%)	0.3% (-5.3 to 5.8%)	0.83

Webtable 5: Mean percentage change in urinary creatinine concentration, albumin concentration and albumin:creatinine ratio in urine with preservative after 1 and 6 month storage, by baseline level of albuminuria

	Mean percentage change (95% CI)		Test for equality of means
	Macroalbuminuria	Normo- or microalbuminuria	
Creatinine			
1 month, -20°C	0.2% (-0.6 to 1.0%)	-0.3% (-0.8 to 0.1%)	0.22
1 month, -40°C	0.5% (-0.7 to 1.8%)	-0.1% (-0.8 to 0.5%)	0.33
1 month, -80°C	1.0% (-0.7 to 2.8%)	-0.4% (-1.3 to 0.5%)	0.16
6 months, -20°C	2.0% (0.9 to 3.0%)	1.6% (1.2 to 2.0%)	0.52
6 months, -40°C	0.2% (-0.5 to 0.9%)	-0.0% (-0.4 to 0.4%)	0.63
6 months, -80°C	0.1% (-0.5 to 0.7%)	0.1% (-0.3 to 0.5%)	0.92
Albumin			
1 month, -20°C	-4.7% (-6.8 to -2.5%)	-11.3% (-15.6 to -7.0%)	0.01
1 month, -40°C	-4.3% (-6.5 to -2.1%)	-0.1% (-2.5 to 2.4%)	0.01
1 month, -80°C	-2.1% (-4.5 to 0.3%)	-2.9% (-7.3 to 1.5%)	0.74
6 months, -20°C	-6.4% (-9.2 to -3.6%)	-29.9% (-38.4 to -21.4%)	<0.001
6 months, -40°C	-4.1% (-5.8 to -2.5%)	-1.5% (-4.3 to 1.3%)	0.11
6 months, -80°C	-1.0% (-2.9 to 1.0%)	0.0% (-3.2 to 3.3%)	0.60
Albumin:creatinine ratio			
1 month, -20°C	-4.8% (-7.0 to -2.6%)	-10.9% (-15.3 to -6.6%)	0.01
1 month, -40°C	-4.6% (-6.7 to -2.5%)	0.0% (-2.5 to 2.5%)	0.005
1 month, -80°C	-2.9% (-4.9 to -0.8%)	-3.2% (-7.6 to 1.2%)	0.90
6 months, -20°C	-8.3% (-11.1 to -5.4%)	-31.0% (-39.3 to -22.6%)	<0.001
6 months, -40°C	-4.4% (-6.3 to -2.6%)	-1.4% (-4.3 to 1.4%)	0.08
6 months, -80°C	-1.2% (-3.3 to 0.8%)	-0.0% (-3.3 to 3.2%)	0.54

Webtable 6: Sources of variation for log urinary albumin:creatinine ratio resulting from pre-analytical handling conditions, assay imprecision, within individual and between individual variation, and their effect on sample size calculations for a hypothetical albuminuria based trial

(a) Sources of variation in log urinary albumin:creatinine ratio (uACR); combined from several studies					
	Within-individual* (from SHARP)	Between-individual* (from SHARP)	Assay imprecision [§] (from laboratory QC)	Pre-analytical handling [†] (from present study)	
				With preservative	Without preservative
SD	0.61	0.90	0.02	0.07	0.10
Mean	6.5	6.5	3.3	4.7	4.6
CV (%)	9	14	0.8	1.4	2.2
(b) Effect on sample size calculations for a hypothetical albuminuria based trial					
Sources of variation				Hypothetical trial sample size [¶]	
Within- and between-individual and assay imprecision without pre-analytic handling variation				188	
Within- and between-individual, assay imprecision and pre-analytical handling (with preservative)				188	
Within- and between-individual, assay imprecision and pre-analytical handling (without preservative)				190	

* Within-individual and between-individual CVs for 1 spot measurement derived from a one-way analysis of variance for 192 CKD patients randomized into a large clinical trial with two urine samples within 12 months and a uACR of at least 150 mg/g at baseline.¹

§ Analytical CVs calculated from two levels of third party quality control material that were measured repeatedly over the time period taken for sample analysis on a Beckman-Coulter DxC800 clinical chemistry analyser.

† Pre-analytical CVs (i.e., variation introduced by different sample collection methods) calculated from the results of this study for samples stored for 4 days @18°C.

¶ The sample size required for a hypothetical trial based on all sources of variation considered together, comparing two treatments using ANCOVA with assumption of 80% power (2p=0.05) to detect a 35% absolute reduction in log uACR (i.e., a relative reduction of 30% in uACR), assuming correlation of 0.6 between successive log uACR measurements.

References

1. Baigent C, Landray MJ, Reith C, et al. The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomised placebo-controlled trial. *Lancet* 2011; **377**(9784): 2181-92.

