Albuminuria: more than a renal risk marker?

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Comparison of urine collection methods for albuminuria assessment in young children

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Abstract

Background

Cotton wool or pantyliners placed in a diaper can be used as urine collection devices for albuminuria measurements in young, not continent children. We tested a new collection method (PeeSpot®) for its analytical performance, and compared it with the pantyliner technique.

Methods

Eighty-one urine samples with a wide range of albuminuria were pipetted on the pantyliner and PeeSpot in duplicate. These were incubated for 3 hours at 37°C (simulating the time a toddler wears a diaper), and subsequently 72 hours at room temperature (simulating transport to a central laboratory). Urine was extracted by centrifugation and albumin concentration (U_{Ac}) was measured. U_{AC} measured by the two methods was compared with U_{AC} in an unprocessed reference aliquot stored for 75 hours at 4°C. Bias (mean percentage U_{AC} difference between test and reference), precision (interquartile range of the U_{AC} difference) and accuracy (proportion of samples within 30% of reference U_{AC}) were calculated.

Results

Median U_{AC} in the reference aliquot was 66.0 mg/L [25^{th}-75^{th} percentile 25.0 – 211.0], pantyliner 32.0 mg/L [4.7 - 165.0; P<0.001 vs reference], and PeeSpot 61.0 mg/L [27.0 - 216.0; P=0.84 vs reference]. Bias, precision and accuracy in pantyliner were -34.2%, 31.3 mg/L and 48.1%; in PeeSpot 3.3%, 5.0mg/L and 96.3%. Passing-Bablok regression and Bland-Altman plot showed an underestimation for the pantyliner but not for the PeeSpot.

Conclusion

The PeeSpot is an accurate and precise tool for collecting urine for albumin measurement in young children and should be preferred over the alternative cotton wool collection technique.

Introduction

Microalbuminuria has been established as a risk marker for cardiovascular and renal disease.[1-6] In the general adult population albuminuria levels differ substantially between individuals and microalbuminuria is prevalent in approximately 7% of the individuals.[7] We recently showed that albuminuria levels show a wide variability between toddlers and that microalbuminuria is also present in approximately 7% of the toddler population.[8]

As opposed to adults, the collection of urine for albumin measurements in toddlers is cumbersome. Modern diapers are extremely efficient in retaining urine, thus old fashioned cotton wool or modern pantyliners are often used to help recovering the urine. This technique is not only 'messy' but does also not result in reproducible results.[9,10] An alternative was found in taped plastic bags which are frequently used in clinical practice. However these have the disadvantage that they detach easily and appear to be uncomfortable.[9] Recently, a novel device for collecting urine in children has been developed, so-called PeeSpot (Figure 1), which potentially allows for more reliable and easy to use measurements. In this study we compared the analytical performance of the pantyliner method with the PeeSpot method.

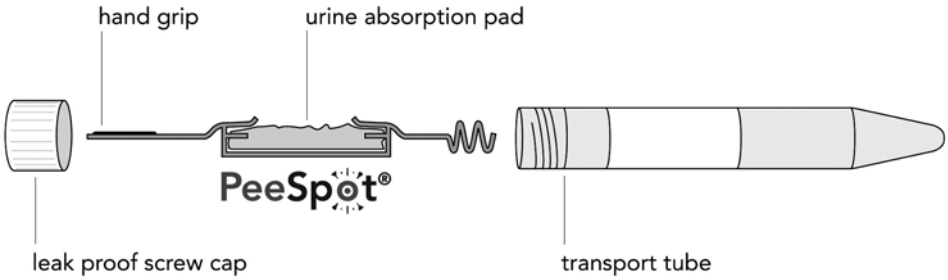


Figure 1. The PeeSpot system. Urine absorption pad is placed in diaper and removed after voiding using the hand grip. Hand grip with the urine absorption pad is placed in the transport tube, closed with the screw cap.

Methods

Urine samples were collected from children who visited the outpatient clinic in the department of pediatric nephrology of the University Medical Center Groningen. Samples were processed anonymously and patients and parents gave permission for use of the urine in this study. Ethical approval was not needed for this study, as determined by The Medical Ethics Committee of the University Medical Center Groningen in the Netherlands.

For the method comparison, the pantyliner method and the PeeSpot method (Hessels+Grob, Lieren, the Netherlands) are compared to a reference (referred to as 'standard method'). Urine samples were pipetted on a pantyliner and PeeSpot in

duplicate. The pantyliner and PeeSpot were stored for 3 hours in a stove at 37°C to simulate the time a toddler wears a diaper. After 3 hours the pantyliner and absorption filter of the PeeSpot were removed from the stove and stored for 72 hours at room temperature to simulate the time for sending the pantyliner or absorption filter to a central laboratory. After 72 hours, the pantyliners were opened, the cotton wool was placed in a centrifuge tube (Qiangen, QIAshredder mini spin column), and the tubes were centrifuged at 10000 rpm for 10 minutes (Rotina 35R). The absorption filters of the PeeSpot were placed within the holder in the tube and centrifuged at 350G for 5 minutes (Rotina 35R). The obtained urine samples were pipetted in cups and urinary albumin concentration (U_{AC}) and creatinine concentration were measured with Roche Modular P (Mannheim, Germany), using respectively the immunoturbimetric assay and the enzymatic method. As reference, we stored a tube of the same urine in the refrigerator at 4°C for 75 hours, and measured albumin and creatinine concentration afterwards.

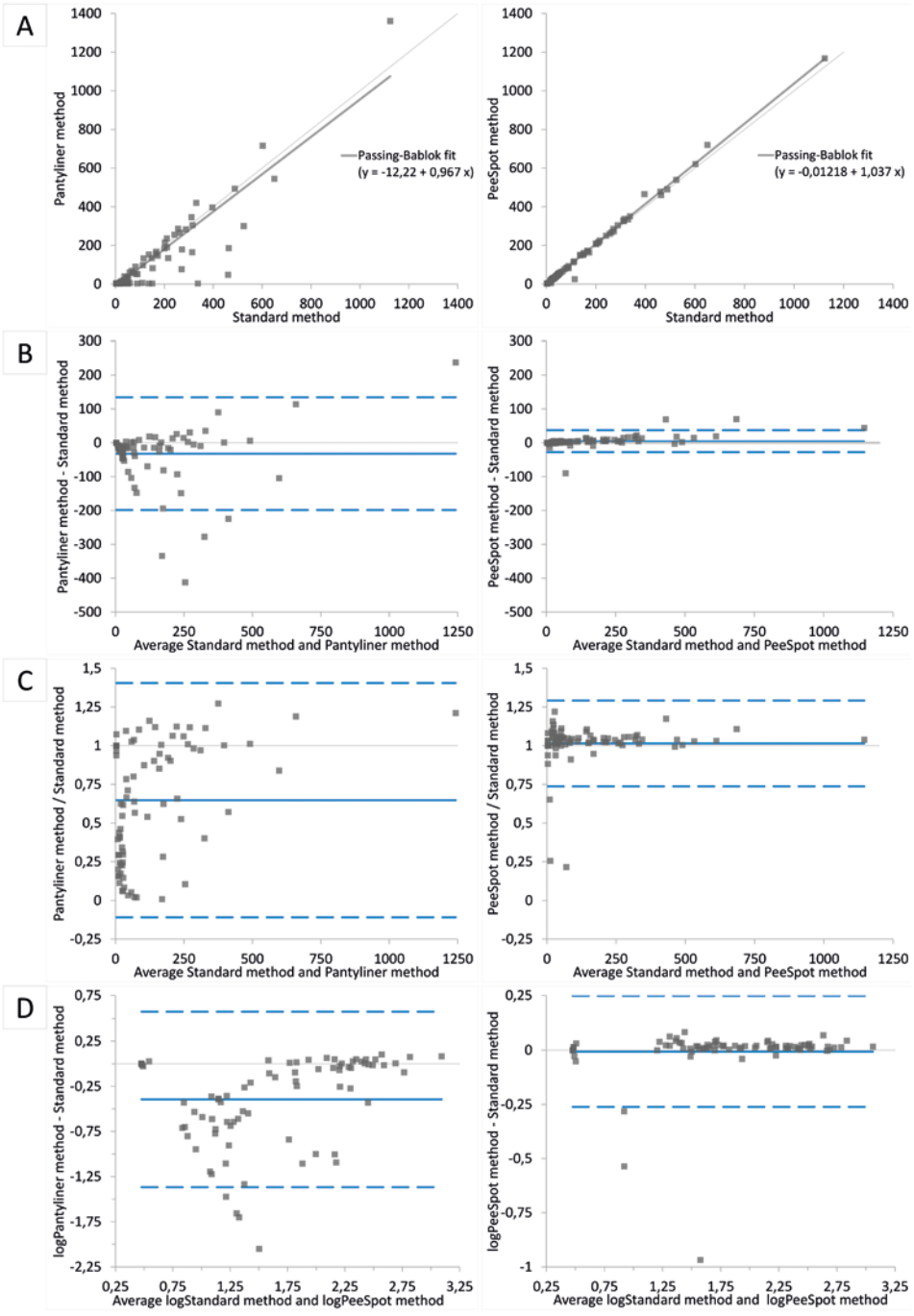
For the validation of the urine collection methods, the National Committee for Clinical Laboratory Standards (NCCLS) guidelines for method comparison were used.[11] According the NCCLS guidelines at least 40 samples across the expected concentration range have to be analyzed for method comparison. In this study 81 urine samples were analyzed in duplicate. The mean of the two measurements was used for analysis. Difference between median albuminuria concentrations was assessed with paired sample T-test on the logarithm of albuminuria concentrations. Performance of the pantyliner and PeeSpot was determined by calculating bias, precision and accuracy as described earlier.[12,13] Bias was defined as the median difference between the standard method and the test method (pantyliner or PeeSpot method). Precision of the difference between the test method and standard method is represented by the interquartile range of the difference between the standard method and test method. Accuracy is calculated as the proportion of values of the test method within ± 30% of the standard method. The range of 30% was chosen based on prior method comparison studies in nephrology in which a 30% range for accuracy was used.[12-14] The repeatability of the standard method, pantyliner method and PeeSpot method were determined by calculating the mean of the difference among duplicate measurements of each method. The sensitivity and specificity for increased albuminuria (U_{AC} ≥20 mg/L) of the pantyliner and PeeSpot were also calculated. Passing-Bablok regression was performed to determine analytical accuracy. Bland-Altman plots were created to assess bias.

Results

Median urinary albumin concentration was 66.0 mg/L [25th-75th percentile 25.0 – 211.0 mg/L], median urinary albumin:creatinine ration (U_{ACR}) was 23.3 mg/mmol [6.6 - 62.7 mg/mmol] in the reference samples. In comparison, median urinary albumin concentration from the pantyliner was 32.0 mg/L [4.7 – 165.0 mg/L, P<0,001 vs reference sample] and from the PeeSpot was 61.0 mg/L [27.0 – 216.0 mg/L, P=0.84]. Median U_{ACR} was 8.8 mg/mmol [1.7 – 38.9 mg/mmol, P<0.001] in the pantyliner and 23.9 mg/mmol [7.1 – 68.3 mg/mmol, P=0.53] in the PeeSpot. Table 1 shows the overall analytical performance of the two test methods compared to the standard method. Bias and precision were markedly higher and accuracy lower with the pantyliner compared to the PeeSpot. The Bland Altman plot showed an underestimation of the urinary albumin concentration for the pantyliner method but not for the PeeSpot (Figure 2). Results were similar when U_{ACR} instead of U_{AC} was used (Supplemental figure 1). For the pantyliner, the mean difference was -32.2 mg/L (95% Limit of Agreement (LoA) -198.6 to 134.2 mg/L). For the PeeSpot, the mean difference was 4.8 mg/L (95% LoA -27.6 to 37.3 mg/L). The repeatability of the standard method was 2.6mg/L, of the pantyliner method 35.4 mg/L, and of the PeeSpot method 4.9 mg/L. The sensitivity and specificity for detection of urinary albumin concentration >20 mg/L were 64.2% and 100% for the pantyliner and 100% and 93.3% for the PeeSpot.

Table 1. Analytical performance measures of pantyliner and PeeSpot method versus standard method

Performance measure	Pantyliner	PeeSpot
Urinary Albumin Concentration		
Bias (mg/L)	-14.0	2.5
Bias (%)	-34.2	3.3
Precision (mg/L)	31.3	5.0
Precision (%)	71.2	4.3
Accuracy (%)	48.1	96.3
Albumin:Creatinine ratio		
Bias (mg/mmol)	-2.6	0.7
Bias (%)	-33.3	3.4
Precision (mg/mmol)	14.2	1.9
Precision (%)	74.8	6.3
Accuracy (%)	46.9	92.6


A
Pantyliner method
Standard method
Passing-Bablok fit
(y = -12,22 + 0,967 x)
PeeSpot method
Standard method
Passing-Bablok fit
(y = -0,01218 + 1,037 x)
B
Pantyliner method - Standard method
Average Standard method and Pantyliner method
PeeSpot method - Standard method
Average Standard method and PeeSpot method
C
Pantyliner method / Standard method
Average Standard method and Pantyliner method
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Average Standard method and PeeSpot method
D
logPantyliner method - Standard method
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Discussion

In this study we compared two methods for measuring albuminuria in young children. We showed that the recently developed PeeSpot device provides highly accurate and precise measurement results and performs markedly better in measuring urinary albumin compared to the pantyliner method.

Why was the performance of the pantyliner method less than the PeeSpot? When we evaluated all individual measurement results we noted that in 31 (19.1%) samples the concentration measured by the pantyliner was below the detection limit whereas the albumin concentration in the reference samples was far above the detection limit, with albumin concentrations varying from 7.9 to 525 mg/L. In the remaining samples the albumin concentration corresponded well with the reference samples. Obviously, this effect had a big impact on the performance of the pantyliner method. A similar phenomenon has been observed in a study that investigated the recovery of albumin when using cotton balls as a urine collection technique.[15] We have no clear explanation for this effect but it may be possible that albumin binds to the cotton wool in the centrifuge tube and therefore is not present in the final urine after centrifuging. We tested whether this effect could be visualized and quantified by using fluorescent assays or specific protein assays for human albumin but the auto-fluorescent cotton wool of the pantyliner prevented visualization and verification of this possibility.

The poor recovery of albumin in some children using the pantyliner technique may have influenced results of previous studies. In a prior study we reported large differences in albuminuria between young children, and found that the prevalence of microalbuminuria in toddlers equals the prevalence of microalbuminuria in the general adult population. These results may have been biased by the collection technique. However, this bias is not likely to have had a big influence on the conclusions of that study, since, in any case, the binding of albumin to the cotton wool would have resulted in an underestimation of the prevalence of microalbuminuria in children.

Apart from being more accurate and precise, there are several other advantages of the PeeSpot for use in clinical practice. First, the PeeSpot method is easier in its use because felts can be centrifuged instantaneously. In contrast, the pantyliner requires more handling procedures since the cotton wool has to be extracted from the pantyliner first. Moreover, the PeeSpot method is more hygienic as the method allows processing

Figure 2. (on the left) Passing Bablok regression and Bland-Altman plots of pantyliner and PeeSpot method. Panel A: Passing-Bablok regression of pantyline method and PeeSpot method. Panel B-D: Bland-Altman plots of both methods. Thick line: mean; Dotted line: 95% Limit of Agreement. Panel B; average of standard method and test method against absolute difference between test method and standard method. Panel C; average of standard method and test method against ratio between test method and standard method. Panel D; logarithm of average standard method and test method against logarithm of difference between test method and standard method.

of the urine without touching it by hand and is more convenient to use in already potty-trained toddlers than pantyliners.

There are a few limitations to our study. We tried to simulate as close as possible the conditions of urine in a diaper in real life. Nevertheless, we may have missed certain specific circumstances which could have biased our results. We were also unable to find the cause of the poor albumin recovery in some of the pantyliner samples.

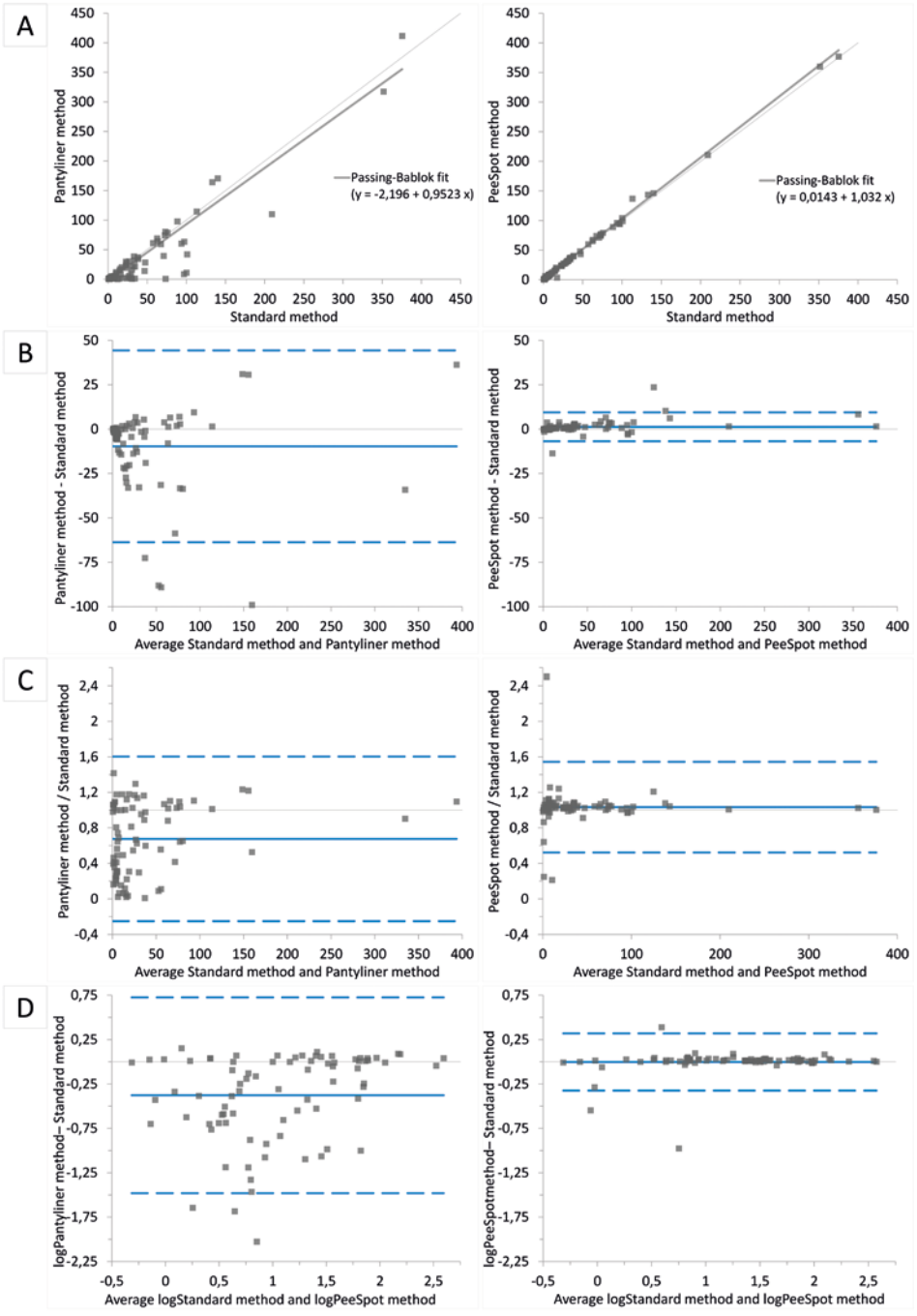
In conclusion, of all the available methods to collect urine in young non continent children, we prefer the PeeSpot as the method is more accurate, precise, and practical in use.

Disclosures

D de Zeeuw has consultancy agreements with the following companies: Abbvie, Astellas, Chemocentryx, Eli-Lilly, Fresenius, Johnson & Johnson, Merck Darmstadt. All honoraria are paid to his institution. HJL Heerspink has consultancy agreements with the following companies: Abbvie, Astellas, Astra Zeneca, Boehringer Ingelheim, Johnson & Johnson, Reata Pharmaceuticals and ZN-Pharma. All honoraria are paid to his institution.

References

1. Keen H, Chlouverakis C, Fuller J, Jarrett RJ. The concomitants of raised blood sugar: Studies in newly-detected hyperglycaemics: II. urinary albumin excretion, blood pressure and their relation to blood sugar levels. Int J Epidemiol. 2014;43(1):11-15.
2. Wachtell K, Palmieri V, Olsen MH, et al. Urine albumin/creatinine ratio and echocardiographic left ventricular structure and function in hypertensive patients with electrocardiographic left ventricular hypertrophy: The LIFE study. losartan intervention for endpoint reduction. Am Heart J. 2002;143(2):319-326.
3. Jarrett RJ, Viberti GC, Argyropoulos A, Hill RD, Mahmud U, Murrells TJ. Microalbuminuria predicts mortality in non-insulin-dependent diabetics. Diabet Med. 1984;1(1):17-19.
4. Parving HH, Mogensen CE, Jensen HA, Evrin PE. Increased urinary albumin-excretion rate in benign essential hypertension. Lancet. 1974;1(7868):1190-1192.
5. Jensen JS, Feldt-Rasmussen B, Borch-Johnsen K, Clausen P, Appleyard M, Jensen G. Microalbuminuria and its relation to cardiovascular disease and risk factors. A population-based study of 1254 hypertensive individuals. J Hum Hypertens. 1997;11(11):727-732.
6. Scheven L, Van der Velde M, Lambers Heerspink HJ, De Jong PE, Gansevoort RT. Isolated microalbuminuria indicates a poor medical prognosis. Nephrol Dial Transplant. 2013;28(7):1794-1801.
7. Hillege HL, Janssen WM, Bak AA, et al. Microalbuminuria is common, also in a nondiabetic, nonhypertensive population, and an independent indicator of cardiovascular risk factors and cardiovascular morbidity. J Intern Med. 2001;249(6):519-526.
8. Gracchi V, van den Belt SM, Kupers LK, Corpeleijn E, de Zeeuw D, Heerspink HJ. Prevalence and distribution of (micro) albuminuria in toddlers. Nephrol Dial Transplant. 2015.
9. Liaw LC, Nayar DM, Pedler SJ, Coulthard MG. Home collection of urine for culture from infants by three methods: Survey of parents' preferences and bacterial contamination rates. BMJ. 2000;320(7245):1312-1313.
10. Macfarlane PI, Ellis R, Hughes C, Houghton C, Lord R. Urine collection pads: Are samples reliable for urine biochemistry and microscopy? Pediatr Nephrol. 2005;20(2):170-179.
11. National Committee for Clinical Laboratory Standards. Method comparison and bias estimation using patient samples; approved Guideline—Second edition. guideline EP9-A2. . 2002;22.
12. Stevens LA, Zhang Y, Schmid CH. Evaluating the performance of equations for estimating glomerular filtration rate. J Nephrol. 2008;21(6):797-807.
13. Tent H, Waanders F, Krikken JA, et al. Performance of MDRD study and CKD-EPI equations for long-term follow-up of nondiabetic patients with chronic kidney disease. Nephrol Dial Transplant. 2012;27 Suppl 3:iii89-95.
14. Witte EC, Lambers Heerspink HJ, de Zeeuw D, Bakker SJ, de Jong PE, Gansevoort R. First morning voids are more reliable than spot urine samples to assess microalbuminuria. J Am Soc Nephrol. 2009;20(2):436-443.
15. Smith GC, Taylor CM. Recovery of protein from urine specimens collected in cotton wool. Arch Dis Child. 1992;67(12):1486-1487


A
Pantyliner method
Standard method
Passing-Bablok fit
(y = -2,196 + 0,9523 x)
PeeSpot method
Standard method
Passing-Bablok fit
(y = 0,0143 + 1,032 x)
B
Pantyliner method - Standard method
Average Standard method and Pantyliner method
PeeSpot method - Standard method
Average Standard method and PeeSpot method
C
Pantyliner method / Standard method
Average Standard method and Pantyliner method
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D
logPantyliner method– Standard method
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logPeeSpotmethod– Standard method
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Supplemental Figure 1 (on the left). Passing Bablok regression and Bland-Altman plots of Pantyliner and PeeSpot method with UACR data. Panel A: Passing-Bablok regression of pantyliner method and PeeSpot method. Panel B-D: Bland-Altman plots of pantyliner method and PeeSpot method. Thick line: mean; Dotted line: 95% Limit of Agreement. Panel B; average of standard method and test method against absolute difference between test method and standard method. Panel C; average of standard method and test method against ratio between test method and standard method. Panel D; logarithm of average standard method and test method against logarithm of difference between test method and standard method.