

# In vitro validation of a method for neonatal urine collection and analysis

Darren Ritchie,<sup>1</sup> Roland Broadbent,<sup>1</sup> Natalie Medicott,<sup>2</sup> David M Reith<sup>1</sup>

**To cite:** Ritchie D, Broadbent R, Medicott N, *et al*. In vitro validation of a method for neonatal urine collection and analysis. *BMJ Paediatrics Open* 2019;**3**:e000482. doi:10.1136/bmjpo-2019-000482

Received 7 April 2019  
Revised 6 May 2019  
Accepted 8 May 2019

## ABSTRACT

**Objective** Urine collection and analysis is important for diagnosis, monitoring of clinical progress, and research in neonates. This study aims to validate a novel methodology for neonatal urine collection, which combines the convenience of cotton ball collection with accurate timing via a urine continence monitor.

**Design** Laboratory model using a combined cotton ball and urinary incontinence monitor method with and without the presence of an impermeable membrane to prevent desiccation.

**Main outcome measures** Accuracy, bias and precision in measurement of urine volume, electrolytes (sodium, potassium, chloride), creatinine and gentamicin. Changes in analyte concentration over time, and evaporative loss of water, were tested using analysis of variance. The effects of time, temperature and humidity were explored using multivariate analysis of variance.

**Results** With the use of an impermeable membrane, sodium concentration increased from a mean (SD) of 3.57% (0.68) at 1 min to 5.03% (0.74) at 120 min. There was no significant change in potassium, chloride or creatinine concentrations. Gentamicin concentration decreased by a mean (SD) of 9.05% (1.37) by 30 min. Multivariate analysis found that absolute change in weight, sodium and chloride were only dependent on duration. Gentamicin concentration was affected by duration, humidity and temperature. Relative evaporative loss was minimal at -0.58% (0.31), and the urinary continence monitor was 100% successful at detecting urination for all time points.

**Conclusions** This novel methodology provides a standardisable and practical method to collect small volumes of neonatal urine for accurate measurement of both urine output and analyte concentrations.

## INTRODUCTION

Urine collections in neonates are important for clinical management and research, but the methods for collecting urine in neonates have not been validated.<sup>1</sup> These collections allow for direct analysis of urine constituents and assist in the measurement of urine output. Although validated in adults, there are few published data validating practical measures for urine collection in neonates.<sup>2</sup> Currently available methods for urine collection in children include perineal urine bags, cotton/gauze inserts, disposable nappies, 'clean-catch' methods and commode inserts. However, despite the large number of

## What is known about the subject?

- ▶ Although validated in adults, there are few published data validating practical measures for urine collection in neonates.
- ▶ The use of a cotton ball is cost-effective, practical and associated with high parental preference in comparison with other urine collection methods.
- ▶ Accurate urine volume measurement is needed for drug clearance estimation, but there are limited data validating the typical method of serial weight measurement of nappies.

## What this study adds?

- ▶ Urine collection from neonates using cotton balls should employ an impermeable membrane between the cotton ball and the nappy.
- ▶ Using cotton balls, urine potassium and creatinine concentrations are reliable, but urine sodium concentration may increase by 5% and gentamicin concentrations may decrease by 10%.
- ▶ Urine continence monitors are feasible for detecting neonatal micturition.

methods, the acquisition of urine from non-toilet-trained neonates presents a variety of challenges and there is little consensus about which is the most appropriate method.<sup>3</sup>

The use of a cotton insert, such as a cotton ball, offers the following advantages. Urine is expressed on-site and cotton can be easily removed directly from the nappy. It is cost-effective, easy to perform and is associated with high parental preference compared with other methods.<sup>1 3-5</sup> There have been several studies on the use of cotton balls for neonatal urine analysis. However, many of these discuss the validity of this method as an assay for measurement of specific environmental chemical exposures. The literature varies in its assessment of the reliability of cotton balls for urine collection, particularly regarding the precision and accuracy of the method on the concentration of studied analytes.<sup>4 6 7</sup> Correspondingly, further analysis



© Author(s) (or their employer(s)) 2019. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

<sup>1</sup>Department of Women's and Children's Health, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand  
<sup>2</sup>School of Pharmacy, University of Otago, Dunedin, New Zealand

## Correspondence to

Dr Darren Ritchie; ritchiedf@gmail.com

has been suggested for collection methods that use absorbent materials, such as cotton balls.<sup>1 3 6</sup>

The most widely used method for measurement of urine volume is the serial weight of nappies before and after urination. Although this is a practical approach, there are limited data to validate urine volume measurements collected in this manner.<sup>4 8 9</sup> Accurate urine volume measurement is needed for drug clearance estimation and methods should be appropriately validated. Ideally, measurement of nappy weight should be timed and measured immediately after urination. Wet nappies can appear dry and clinic staff may be unaware of when urination occurred.<sup>9</sup> There has been no research in the use of urinary continence monitors for timed urine collections and clearance calculations.

Cotton ball methods for collecting urine have been predominantly used on the basis of 'routine practice', rather than a strong base of evidence. If a cotton ball methodology were scientifically validated, it would provide a practical means to acquire important information and alleviate the inherent difficulties in the collection of urine from neonates.<sup>3</sup>

The aim of the present study is to validate a urine collection method for neonates which combines the convenience of cotton ball collection with accurate timing via a urine continence monitor, for the purpose of drug clearance research. Furthermore, given the absence of data, the study aims to determine whether continence monitors have utility in the proposed methodology by providing accurate timing of micturition in neonates and thus allowing the calculation of urinary clearance.

## METHODS

A modified neonatal mannequin was used for this study and was placed into a paediatric incubator (Draeger Isolette C2000). This replicated the thermal environment of an infant in a modern incubator. The neonatal mannequin comprised a doll which was modified to allow thermoregulated water to circulate through the abdomen, perineum and legs with standard stainless-steel plumbing fittings and plastic tubing. The water was thermoregulated to achieve a target skin (surface) temperature of 36.5°C.

### Thermoregulation of the neonatal mannequin

A 1.7-litre 1100 W domestic water kettle was used as the water heater for this study. The kettle was electronically controlled by an external temperature controller box fitted with an Omega CNi3244 temperature controller. The controller was tuned by its proportional integral differential auto-tune function to maintain water temperature at 36.5±0.1°C by pulsing an IDEC Corp. RSSDN-10A solid-state relay with 0 V crossing point-switching. The temperature sensor was an Omega HSTC-TT-KI-24S-1M type K PTFE hermetically sealed thermocouple that was fixed and immersed in the water of the kettle. The heater was thermally insulated using expanded plastic sheeting to prevent heat loss. The water was then continuously

circulated through the compartments of the mannequin by a peristaltic pump connected between the thermoregulation unit and the mannequin.

### Simulated urine collection and analysis

Libero Newborn Premature Nappies were applied to the mannequin, and a cotton ball was introduced to the urinary area. A Wet-Stop3 urinary continence monitor was fastened to the perineal area of the nappy and measurements were made with and without an impermeable membrane, placed between the cotton ball and the nappy. The impermeable membrane consisted of retail-available plastic wrap trimmed to the size of the cotton ball in order to ensure negligible contact with the skin. The urinary continence monitor was connected to an audible alarm to monitor the occurrence of simulated micturition. Urine samples were collected from a healthy human volunteer and electrolyte and creatinine concentrations were measured. Aqueous gentamicin was added to the urine to achieve a level of 8 µg/mL. A syringe was used to introduce a representative volume of 10 mL urine to the nappy over 30 s.<sup>10</sup> After separate introductions of urine, timed collection (1 min, 30 min, 1 hour and 2 hours) of the cotton ball was performed. Fluid was expressed into a standard specimen tube by direct syringe expression. Urine samples were refrigerated at 4°C for less than 24 hours before analysis. Samples were sent to Southern Community Laboratories for measurement of urinary concentrations of creatinine, sodium, potassium, chloride and gentamicin. Laboratory analysis was conducted using a Roche Cobas 8000 analyser (using the Jaffe method for measuring urinary creatinine).

In order to estimate evaporative losses, the nappy together with cotton balls was weighed pre-urination and post-urination, and at each collection time. This was undertaken using scales with a reproducibility of 0.1 mg SD (Mettler AE200).

All experiments were performed in six replicates. Baseline measurements were made prior to introduction of urine into the nappy.

### Statistical analysis

Summary statistics were calculated as relative accuracy ((baseline–recovered)/baseline×100, expressed as mean %), bias (baseline mean–sample mean, expressed as mean difference) and precision (SD/mean, expressed as coefficient of variation). Changes in analyte concentration over time, and evaporative loss of water, were tested using analysis of variance. The effects of duration, humidity and temperature were explored using multivariate analysis of variance (MANOVA) to determine the effect of the environmental variables on the concentrations of the analytes. We tested the continuous variables for normality and correlation; these approximated normal distributions and did not show correlation.

Ethical approval was obtained through the University of Otago Human Ethics Committee (Health) (ref:

**Table 1** Accuracy and precision of analyte recovery from urine introduced into neonate nappies without impermeable membrane and with impermeable membrane

	Without impermeable membrane			With impermeable membrane		
	Absolute change		Relative change	Absolute change		Relative change
	Accuracy (mean)	Precision (CV %)	Accuracy (mean %)	Accuracy (mean)	Precision (CV %)	Accuracy (mean %)
<b>Sodium</b>	mmol/L			mmol/L		
0 min	NA	0.25%	NA	NA	0.30%	NA
1 min	1.420	1.51%	7.43%	1.900	0.67%	3.57%
30 min	4.580	13.74%	21.76%	2.220	0.75%	3.92%
60 min	*	*	*	2.300	0.77%	4.04%
120 min	3.880	*	22.03%	2.740	0.90%	5.03%
<b>Potassium</b>	mmol/L			mmol/L		
0 min	NA	0.49%	NA	NA	0.14%	NA
1 min	-0.256	1.39%	-1.82%	-0.100	0.39%	-0.84%
30 min	0.659	5.16%	3.59%	-0.028	0.30%	-0.81%
60 min	*	*	*	-0.028	0.56%	-0.41%
120 min	1.066	*	7.01%	-0.060	0.41%	-0.37%
<b>Chloride</b>	mmol/L			mmol/L		
0 min	NA	0.65%	NA	NA	0.24%	NA
1 min	0.340	0.82%	1.36%	0.060	0.55%	0.20%
30 min	3.067	7.62%	11.60%	0.320	0.45%	0.70%
60 min	*	*	*	0.580	0.87%	1.27%
120 min	2.700	*	12.84%	0.440	0.63%	1.10%
<b>Gentamicin</b>	µg/mL			µg/mL		
0 min	NA	1.66%	NA	NA	1.13%	NA
1 min	-5.336	17.64%	-53.15%	-0.146	1.56%	-1.80%
30 min	-8.265	41.36%	-81.36%	-0.810	1.63%	-8.49%
60 min	*	*	*	-0.864	3.40%	-9.75%
120 min	-8.192	*	-82.22%	-0.910	1.49%	-9.05%
<b>Creatinine</b>	µmol/L			µmol/L		
0 min	NA	2.14%	NA	NA	1.27%	NA
1 min	-0.200	3.52%	-1.03%	-0.020	0.91%	0.01%
30 min	0.207	5.52%	5.92%	-0.020	0.72%	-0.46%
60 min	*	*	*	0.040	1.33%	-0.26%
120 min	0.140	*	3.85%	-0.120	1.63%	-0.37%

\*Refers to lack of data due to sample desiccation. CV, coefficient of variation; NA, not available.

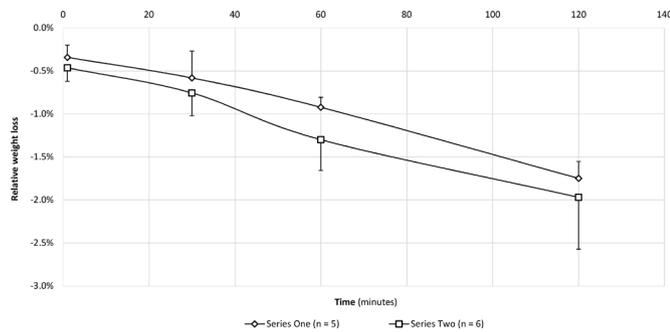
H16/135). This research was done without public or patient involvement.

## RESULTS

Data were available for 35 of the 48 collected samples. When an impermeable barrier was used, all of the cotton balls contained sufficient sample for analysis. Conversely, in the absence of an impermeable barrier, only 11 of 24 cotton balls contained sufficient sample for analysis at the baseline collection. Furthermore, by the 30 min time point, four out of six cotton balls were desiccated and there was insufficient sample for analysis, indicating this method is not suitable for collecting specimens after this time (table 1). There was also greater inaccuracy, due to net loss of water from the

sample over time, with an increase in the concentration of sodium, potassium, chloride and creatinine. However, the changes in concentrations of these solutes were not proportional, and the ratio of sodium to creatinine changed over time. There was also a decreased recovery of gentamicin, relative to the samples with an impermeable membrane, in those collected immediately after the sample was added to the cotton ball.

When an impermeable barrier was used, there was an increase in the concentration of sodium that increased from a mean (SD) of 3.57% (0.68) at 1 min to 5.03% (0.74) at 120 min, indicating either a small net loss of water or some leaching of sodium from the cotton wool. The precision ranged from 0.30% to 0.90%, indicating



**Figure 1** Weight loss of nappy with urine in cotton balls, reflecting fluid loss, over time for nappies without an impermeable membrane (diamonds, n=5) and with an impermeable membrane (squares, n=6). Mean data are plotted and error bars represent the SD.

this change was predictable. There were small decreases in potassium and chloride concentrations, which were within the margin of error for the assay. There was a small decrease in creatinine concentration, with a maximum mean (SD) change of  $-0.37\%$  (2.0) at 120 min and with a precision of up to 1.63%. Gentamicin concentration decreased by a mean (SD) of 9.05% (1.37) by 30 min. This suggests binding of gentamicin to the cotton wool. However, this decrease in concentration was predictable, as the precision was at most 3.4%.

Multivariate analysis of variance was not conducted on the data for series 1 due to paucity of data (a result of desiccation). The MANOVA for samples collected when the impermeable membrane was used, including time since urine introduction, humidity and temperature, found that absolute change in nappy weight, sodium and chloride were only dependent on duration. Creatinine concentration was only dependent on baseline creatinine levels. Gentamicin concentration was affected by duration, humidity and temperature.

The results of the serial weight measurement pre-urination and post-urination for each time point is represented in figure 1. The relative weight loss at 30 min was minimal at  $-0.58\%$  (0.31) and  $-0.76\%$  (0.27) without and with the membrane, respectively.

The urinary continence monitor was 100% successful at detecting urination for all 48 time points. The operation of the monitor could also be carried out with ease, being fastened to the perineal surface of the nappy with the inbuilt clip.

## DISCUSSION

The method of urine collection tested in the present study delivered reliable results when an impermeable barrier was used. When an impermeable barrier was used, the method is within acceptable bounds for measuring sodium, potassium, chloride and creatinine. However, consideration may need to be made for adjusting the concentration of sodium and whether sodium is leached from the cotton wool. Environmental temperature and humidity, within the ranges experienced by neonates in an incubator, did not influence

sodium, potassium and chloride concentration for up to 120 min. The multivariate analysis was consistent with other studies in that delay in measurement did not have a significant role in the recovery of creatinine from the sample.<sup>6</sup> In the case of gentamicin, the changes were observed at 30 min, so it is possible that immediately collecting the sample would provide more reliable results. Gentamicin recovery over time was influenced by environmental temperature and humidity. Hence, using this technique for drug studies may require extraction techniques, from the cotton wool, and also validation for each drug and its metabolites. Although predictable, this is consistent with the literature in regard to the variable analyte recovery from the cotton matrix for specific analytes.<sup>6-11</sup> Consequently, we would recommend that other analytes be validated before cotton wool is used as a medium for urine collection.

The urinary continence monitor was effective for detecting urine introduction into the nappy but is unlikely to make a difference for the measurement of sodium, chloride and gentamicin concentrations because, in the presence of an impermeable barrier, the changes occur within 1 min. However, continence monitors may prevent the collection of 'superimposed' urinations from a single sample and allow more efficient recognition of micturition for analytes in which recovery is less time dependent. Accurate timing of urine output is essential in any measurement of urinary clearance, and the accuracy shown for the continence monitor is sufficient for this purpose. Furthermore, continence monitors still have an important role in the prevention of faecal contamination of urinary samples. Further investigation is needed to determine whether our results are generalisable to a patient population, where neonates are active and void volumes are variable.

The present study indicates that, in the conditions studied, evaporative loss was not a significant factor. Previous studies have indicated evaporative loss from nappies over 120 min can be up to 16% in an incubator without phototherapy, 20% in an incubator with phototherapy and 21% under a radiant warmer.<sup>9</sup> However, evaporative loss is highly influenced by humidity, and in modern incubators, with 60% to 85% humidity, there is little evaporative loss.<sup>8</sup>

The effect of the impermeable membrane on water transfer from the cotton ball to the nappy was highly significant. All the specimens collected with a membrane in place had sufficient urine to allow analysis, and of those collected without the impermeable membrane, 13 out of 24 were so desiccated that there was insufficient specimen for analysis.

In the literature, there are two main methods of urinary fluid expression from cotton balls. The first technique, as in the present study, is the direct expression of the urine from the cotton ball using syringe compression.<sup>1-6</sup> The second technique is the centrifugation of the cotton matrix to express the urinary fluid.<sup>12</sup> There has been no comparison in the literature as to the relative accuracy of either method. An alternative method for urine collection is direct expression of urinary fluid disposable nappies. Disposable nappies

normally contain super-absorbable polymers such as polyacrylate, which make extraction of urine complicated. However, unlike cotton balls, a significantly greater volume of urine is potentially expressible and available for analysis. Consequently, there has been investigation into solutions that allow the release of target metabolites from polymers for measurement.<sup>13</sup> Although the results of these investigations are encouraging, there remains a significant structural and polymer variation in nappy brands, presenting significant difficulties in standardisation. For example, one study found that certain nappy materials selectively adsorbed creatinine.<sup>14</sup> Additionally, Goodpaster *et al* determined that nappy brands had distinct contaminant profiles that could influence the results of metabolomic studies of urine from newborn babies.<sup>11</sup> In their study, minimal contamination was detected in 'cotton ball alone' samples, and that 'nappy plus cotton ball' samples had less contamination than 'nappy only' samples. We suggest that the incorporation of an impermeable membrane represents a significantly more practical method than standardisation of nappy matrices to reduce contaminant levels or analyte leaching. A cotton ball, although less useful in older children, provides a standardisable and practical method to collect small volumes of neonatal urine for accurate measurement.

The stability of solutes in the cotton matrix is essential for accurate measurement.<sup>13</sup> Despite this, there has been no investigation into the effects of different sample storage (ie, temperature and sample handling). In the present study, the protocol for sample handling would be readily reproducible in most neonatal intensive care units. Furthermore, there is a paucity of evidence in the literature on other factors that may change solute recovery, such as nappy creams/ointments or fluid lost via the skin with respect to skin thickness.<sup>1</sup>

The main strengths of this study come from the robust collection of data. We had the ability to make measurements at a very small scale with very sensitive measurements. Additionally, radiant body heat and continence monitoring has not been studied extensively in the literature. Furthermore, we included sufficient replicates in the protocol for laboratory validation, as many other studies have had small sample sizes.

The main limitation of this study arises from being an *in vitro* model. Consequently, there is uncertainty to whether the results would translate to an *in vivo* model. Another limitation was that we adapted a plasma gentamicin assay for measurement of urine. The results of gentamicin were expressed as relative changes with respect to the baseline measurements.

## CONCLUSION

Urine collection from neonates using cotton balls should employ an impermeable barrier between the cotton ball and the nappy. Using cotton balls, urine potassium and

creatinine concentrations are reliable, but urine sodium concentration may increase by up to 5% and gentamicin concentrations may decrease by up to 10%. Urine continence monitors are feasible for detecting neonatal micturition.

**Contributors** DR, RB, NM and DRM designed the research, which was conducted by DR in terms of collection of data and drafting of the manuscript. All authors contributed to interpretation of data and revision of manuscript.

**Funding** The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

**Competing interests** None declared.

**Patient consent for publication** Not required.

**Ethics approval** Ethical approval was obtained through the University of Otago Human Ethics Committee (Health) (ref: H16/135).

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** All data relevant to the study are included in the article or uploaded as online supplementary information.

**Open access** This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

## REFERENCES

- Burke N. Alternative methods for newborn urine sample collection. *Pediatr Nurs* 1995;21:546–9.
- Barr DB, Wang RY, Needham LL. Biologic monitoring of exposure to environmental chemicals throughout the life stages: requirements and issues for consideration for the National Children's study. *Environ Health Perspect* 2005;113:1083–91.
- Lee EJ, Arbuckle TE. Urine-sampling methods for environmental chemicals in infants and young children. *J Expo Sci Environ Epidemiol* 2009;19:625–33.
- Roberts SB, Lucas A. Measurement of urinary constituents and output using disposable napkins. *Arch Dis Child* 1985;60:1021–4.
- Liaw LC, Nayar DM, Pedler SJ, *et al*. Home collection of urine for culture from infants by three methods: survey of parents' preferences and bacterial contamination rates. *BMJ* 2000;320:1312–3.
- Hu YA, Barr DB, Akland G, *et al*. Collecting urine samples from young children using cotton gauze for pesticide studies. *J Expo Anal Environ Epidemiol* 2000;10:703–9.
- Urinary tract infection in under 16s: diagnosis and management, 2017. Available: <https://www.nice.org.uk/guidance/CG54/> [Accessed 22 Aug 2017].
- Oddie S, Adappa R, Wyllie J. Measurement of urine output by weighing nappies. *Arch Dis Child Fetal Neonatal Ed* 2004;89:180F–1.
- Gouyon JB, Sonveau N, d'Athis P, *et al*. Accuracy of urine output measurement with regular disposable nappies. *Pediatr Nephrol* 1994;8:88–90.
- Wahl EF, Lahdes-Vasama TT, Churchill BM. Estimation of glomerular filtration rate and bladder capacity: the effect of maturation, ageing, gender and size. *BJU Int* 2003;91:255–62.
- Goodpaster AM, Ramadas EH, Kennedy MA. Potential effect of diaper and cotton ball contamination on NMR- and LC/MS-based metabolomics studies of urine from newborn babies. *Anal Chem* 2011;83:896–902.
- Calafat AM, Needham LL, Silva MJ, *et al*. Exposure to di-(2-ethylhexyl) phthalate among premature neonates in a neonatal intensive care unit. *Pediatrics* 2004;113:e429–34.
- Hu Y, Beach J, Raymer J, *et al*. Disposable diaper to collect urine samples from young children for pyrethroid pesticide studies. *J Expo Anal Environ Epidemiol* 2004;14:378–84.
- Mock DM. Rayon balls and disposable-diaper material selectively adsorb creatinine. *Am J Clin Nutr* 1992;55:326–30.