

# A Novel Method for Isolating and Quantifying Urine Pathogens Collected from Gel-Based Diapers

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**Background.** Given that children often present to physicians with a wet gel-based diaper, a method for using this diaper for a urine specimen was studied.

**Methods.** A blinded clinical laboratory trial was conducted in the microbiology laboratory. The sampling technique involved the use of oval gynecologic forceps as a template and sterile scissors to cut out samples of diapers. Each diaper sample was then vortexed in 20 mL of sterile saline and the supernatant quantitatively cultured. Diaper sample supernatant cultures with simulated infected urines (suspensions of *Escherichia coli*) were used.

**Results.** Weight measurements of the diaper samples soaked with 20 mL or more of saline yielded reproducible results (0.703 g; 95% confidence interval [CI], 0.52

to 0.88 g). Culture results (colony-forming units per liter [CFU/L] of supernatant) from diapers soaked with 25 mL of various concentrations of *E coli* ( $10^6$ ,  $10^7$ ,  $10^8$ ,  $10^9$  CFU/L) showed excellent correlation with the inoculum used, and no effect of a 2-hour delay in culturing the wet diapers.

**Conclusions.** Our technique provides accurate and reliable estimates of bacterial concentrations in the usual range for infected urine from gel-based diapers soaked with solutions of *E coli*. Further evaluation of clinical implementation is needed.

**Key words.** Urine; bacteria; *Escherichia coli*; pathogens; infection; diapers; infant.

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Urinary tract infections (UTIs) in children can present with a diverse array of clinical manifestations. Consequently, UTIs must be considered in the differential diagnosis of a variety of disorders. Substantiation requires the collection of urine for quantitative culture. For infants and young children who may be unable or unwilling to cooperate in the urine-collection process, midstream urine collection may be difficult or impossible. For these patients, urine is usually collected by means of adhesive bags, urethral catheterization, or suprapubic aspiration.

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The use of bags has several drawbacks: (1) failure to adhere; (2) perineal discomfort and trauma (especially in children with sensitive skin); and (3) a high incidence of mixed cultures reflecting a contaminated sample.

Ahmad et al<sup>1</sup> have shown that urine can be collected for culture from disposable diapers by extracting it from the wet fibers. They demonstrated good agreement in bacterial counts between urine collected this way and urine collected in bags. Their study also suggests that the contamination rate with this procedure may be lower than with the bag specimens. In an assessment of the reliability for microbiological analysis of urine collection from disposable diapers of elderly women with severe urinary incontinence, Belmin et al<sup>2</sup> found that it was a simple and reliable method for routine analysis. Contamination or bacterial concentration change over time was not observed.

A major limitation of their technique is that it is not applicable to gel-based diapers. This type of diaper dominates the markets of North America and some areas in



Europe, such as Sweden.<sup>3</sup> Disposable diapers contain cottonwool fibers from which urine specimen can be extracted by compressing the wet fibers. Unfortunately, with gel-based ultra-absorbent diapers urine cannot be collected in the same manner. We have, therefore, developed a method for culturing urine from this type of diaper. We present preliminary results of the laboratory developing phase.

## Methods

In culturing urine, it is necessary to identify not only the infectious agent in the specimen but also its concentration. Therefore, because it is impossible to extract urine from ultra-absorbent diapers using the compression method, a standardized method for sampling a fixed amount of urine from this type of substance is needed.

A standardized method of culturing gel-based diapers is as follows:

1. Huggies brand medium boys' diapers are bisected along the long axis with sterile fine dissecting scissors
2. A 10-inch ring gynecologic forceps is applied as a template to get a sample of the ultra-absorbent substance 6 to 18 cm from the diaper edge
3. The perimeter of the sample is then gently wiped with the back of the scissor blade to remove any loosely adherent gel particles, producing a diaper sample within the oval. We used this forceps to obtain a standard fixed sample.
4. The clamp is then loosened and the diaper sample dropped into 20 mL of saline solution prepared in advance in a sterile container.

For culture, the container is capped and vortexed for 10 seconds, after which 500  $\mu$ L of supernatant is removed and spread on appropriate agar plates. These are incubated at 37°C and read after 24 hours. Equipment required for this procedure includes disposable gloves, sterile oval gynecologic forceps, sterile sharp-nosed dissecting scissors, sterile container with 20 mL of sterile saline solution, vortex mixer, 500- $\mu$ L pipette, and standard agar plates used for urine cultures. Since we were attempting to establish a standard method to obtain a standard sample, we evaluated whether the dry or wet sample weight varies according to distance from edge of the diaper.

As a preliminary step, dry and wet diaper samples were obtained from various distances along the long axis of the diaper, with the anterior-most edge of the diaper representing a distance of zero and weighed on an analytical balance. This allowed for the assessment of differences in gel distribution that might occur in the sampled area.

Table. Diaper Sample Weight As a Function of the Volume of Saline and the Distance from the Diaper Edge

Variables	Weight (g)*	Standard Deviation
Distance from diaper edge (cm)		
Dry sample		
3.0	0.21	0.01
5.5	0.26	0.01
8.0	0.32	0.02
10.5	0.30	0.02
13.0	0.31	0.01
15.5	0.31	0.01
18.0	0.32	0.02
20.5	0.25	0.02
Wet sample†		
6.0	1.04	0.11
10.0	1.00	0.07
14.0	1.01	0.11
18.0	0.92	0.25
Volume of saline poured into diaper (mL)		
10.0	0.96	0.25
20.0	1.02	0.08
25.0	1.01	0.09
30.0	1.00	0.09

\*Average of four samples at each position and with each volume.

†Average data from 20, 25, and 30 mL saline only.

To obtain wet diaper samples, varying volumes of water (10, 20, 25, and 30 mL) were emptied into the midline of the diaper before the various diaper samples were obtained.

Mock infected urine was generated using a pure culture of *Escherichia coli* mixed in sterile normal saline. The concentration of the stock solution was obtained by comparison with a McFarland 0.5 turbidity standard with serial 10-fold dilutions performed to generate solutions of  $10^6$ ,  $10^7$ ,  $10^8$ , and  $10^9$  CFU/L.

As a first experiment, 25 mL of these solutions was placed into duplicate diapers and two samples from each diaper were obtained in an unblinded manner immediately and 2 hours later. A second blinded experiment was conducted, using 25 mL each of eight coded solutions containing  $10^5$  to  $10^9$  colony-forming units per liter of *E coli*. Each inoculum was poured into two diapers, each of which was sampled twice for culture.

Correlation was performed according to the least-squares method,<sup>4</sup> with the *P* value reflecting the two-tailed probability that the correlation slope is not equal to zero.

## Results

The dry diaper sample weights (Table) show a decline near the front and back of the anterior half of the diaper. The mean of the dry weights along the long axis of the



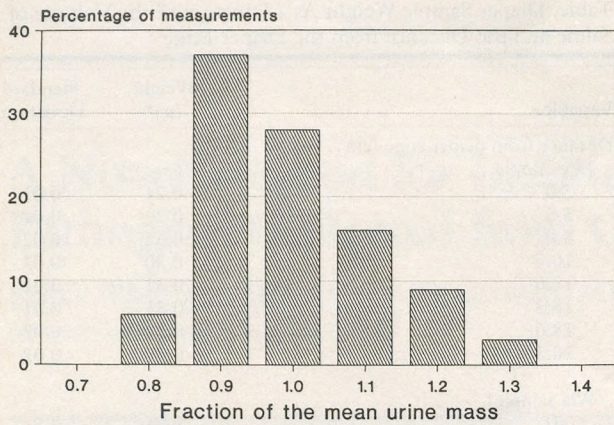


Figure 1. Distribution of measurements of fluid mass in the wet diaper samples, as calculated by subtracting the average dry diaper sample weight from the actual wet diaper sample weight (N=16).

diaper between 5.5 to 18 cm from the edge were consistent, with an average weight of 0.284 g (standard deviation (SD), 0.026). The wet diaper weights in the Table show that the mean sample weight is lower and more variable when a 10-mL volume is used. It is likely that this difference is due to nonuniform gel saturation with small volumes of liquid. Pooling the data from diapers soaked with 20 to 30 mL demonstrates that the weight of diaper samples taken between 6 and 18 cm from the edge is relatively constant. Therefore, for all subsequent experiments, diaper samples were taken between 6 and 18 cm from the diaper edge, and liquid volumes of 25 mL were used.

Subtracting the dry from the wet diaper mass allows calculation of the amount of liquid in the diaper sample. The average liquid mass in a wet diaper sample was 0.703 g (SD, 0.091). The liquid mass measurements show a skewed normal distribution around the mean (Figure 1) with values beyond two standard deviations, accounting for fewer than 26% of the specimens.

Figure 2 shows the results of the first experiment with bacterial solutions. In this experiment two samples from each of two identical diapers were obtained, one immediately and the other 2 hours later. No effect of a 2-hour delay was seen. Results of the second blinded experiment with coded blind bacterial solutions are shown in Figure 3. There was excellent correlation between the diaper supernatant cultures and the bacterial concentration in the solution used to wet the diaper ( $r=.99$ ;  $P<.001$ ).

The correction factor for determining the original bacterial concentration was derived by calculating the formula for the correlation line, which is shown in Figure 3.

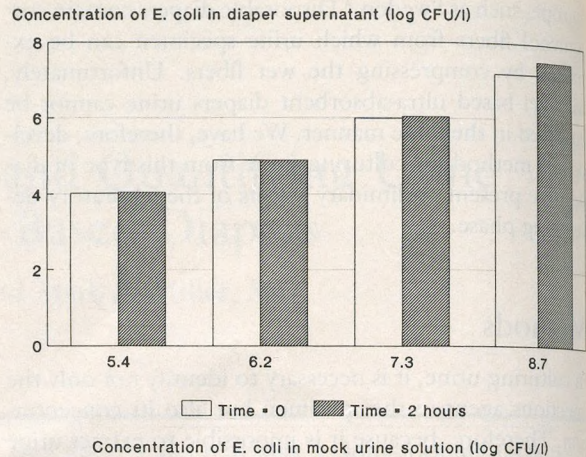


Figure 2. Effect of a 2-hour delay in obtaining diaper samples on the yield of diaper sample cultures. Log denotes logarithm; CFU/L, colony-forming units per liter.

## Discussion

The data from these experiments with bacterial solutions demonstrate that this novel technique using liquid collected from gel-based diapers can closely predict the concentration of *E. coli* in solutions of mock infected urine. The degree of variability between the predicted and actual bacterial concentrations is clinically insignificant because the diagnosis of a UTI is usually based on colony concentrations spanning orders of magnitude. Although the sample is small, the high correlation coefficient ( $r=.99$ ) provides sufficient basis to proceed from the experimental laboratory phase to the clinical phase. The experimental

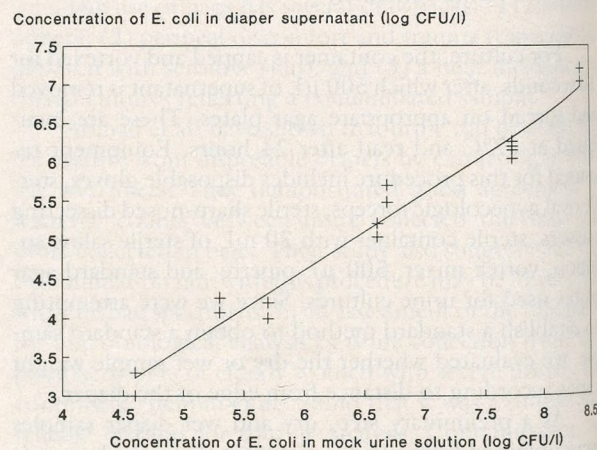


Figure 3. Correlation of cultures of diaper sample supernatant with mock infected urine, ie, suspensions of *Escherichia coli* in sterile saline. The formula for the correlation line is:  $y=1.43+0.98x$ . The correlation coefficient ( $r$ ) is .99 ( $P<.001$ ). Log denotes logarithm; CFU/L, colony-forming units per liter.



protocol used restricts immediate generalization to the clinical setting for several reasons.

First, all the diapers used in these experiments were Huggies, size medium, for boys. It is not known if the gel distributions and concentrations in other brands or sizes are different. Second, the data show the ability of *E coli* to survive exposure to the gel and to be readily extractable from the diaper. Although this is a potential limitation, it seems unlikely that other urine pathogens would behave differently. Although the diaper components were not found to be toxic to *E coli*, the effect of longer contact times is unknown. In 1989, Lee Wong and Assenheimer Downs<sup>5</sup> showed differential effects of various fibers on the growth characteristics and toxin production of *Staphylococcus aureus*. Third, the volume of urine voided by infants with UTI is unknown. However, it is important to remember that the bladder capacity of infants is surprisingly large. Term newborns have 50 to 60 mL of bladder capacity, and this increases to 100 mL by the end of the first year.<sup>6</sup> It seems likely that there is frequent bladder emptying in infants with UTI. However, since weight measurements suggest that even 10 to 20 mL will yield reproducible results with our technique, this should not represent a clinical limitation.

Several potentially relevant issues are not addressed by these experiments. Diapers worn by infants are in direct contact with the perineum and could become contaminated. Clearly, gross fecal soiling would interfere with our technique. Ahmad et al<sup>1</sup> demonstrated that diaper-collected cultures were no more often contaminated than those collected by urine bags. Their technique uses "deep" diaper fibers that might be less susceptible to contamination. Similar findings were observed in a recent study of elderly incontinent women.<sup>2</sup> The diapers used in our study have a "liner" that can be removed from the sample, something we did not do. If contamination proves to be a problem in clinical studies, this additional step could be added.

It can be argued that the most accurate method of obtaining urine for culture in infants is by means of suprapubic aspiration. Many primary care physicians may be uncomfortable performing this invasive procedure. A recent study of suprapubic aspiration in a children's hospital showed that the use of portable ultrasound increased the yield of successful taps from 52% to only 79%.<sup>7</sup> Clearly, alternative methods that are both less invasive and more successful are still required.

Studies investigating this novel technique of diaper culture in the clinical pediatric setting are currently under way. These future studies will evaluate whether the technique is simple enough for use in a busy office. We assume that not more than 3 to 5 minutes will be needed to collect the specimen.

If successful, the method might also be applicable to a diaper-wearing geriatric population, which would alleviate the need for catheterization.

#### References

1. Ahmad T, Vickers D, Campbell S, Coulthard MG, Pedler S. Urine collection from disposable nappies. *Lancet* 1991; 338:1077-8.
2. Belmin J, Hervias Y, Avellano E, Oudart O, Durand I. Reliability of sampling urine from disposable diapers in elderly incontinent women. *J Am Geriatr Soc* 1993; 41:1182-6.
3. Bjorklund LJ. Urine collection from disposable nappies [letter]. *Lancet* 1991; 338:674-6.
4. Colton T. *Statistics in medicine*. Boston, Mass: Little, Brown and Co Inc, 1974:189-206.
5. Lee Wong AC, Assenheimer Downs S. Investigation by improved syringe method of effect of tampons on production in vitro of toxic shock syndrome toxin 1 by *Staphylococcus aureus*. *J Clin Microbiol* 1989; 27:2482-7.
6. Currarino G. The genitourinary tract. In: Solveman FN, Kuhn JP, eds. *Essentials of Caffey's pediatric x-ray diagnosis*. Chicago, Ill: Yearbook Medical Publishers, Inc, 1990:653-733.
7. Gochman RF, Karasic RB, Heller MB. Use of portable ultrasound to assist urine collection by suprapubic aspiration. *Ann Emerg Med* 1991; 20:631-5.