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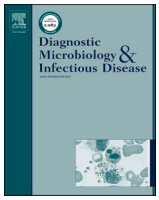

Original Article

Diaper-based diagnostics: A novel non-invasive method for urine collection and molecular testing of uropathogens

Tyler Vine [a,b], Rob E. Carpenter [a,c,*], Debbie Bridges [d]

[a] Department of Research, Advanta Genetics, 10935 CR 159, Tyler, TX 75703, United States
[b] Miller School of Medicine, University of Miami, 1600 NW 10th Ave, Miami, FL 33136, United States
[c] Soules College of Business, The University of Texas at Tyler, 3900 University Boulevard, Tyler, TX 75799, United States
[d] Health Science Center, The University of Texas at Tyler, 11937 US-271, Tyler, TX 75708, United States

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ABSTRACT

Urinary tract infections (UTIs) present a global diagnostic challenge, especially in populations where midstream urine collection is impractical. This study evaluates sodium polyacrylate-based diapers as a non-invasive matrix for urine collection, coupled with quantitative PCR (qPCR) for uropathogen detection. Phase I involved 17 samples (7 contrived, 10 from diaper-wearing volunteers), and Phase II analyzed 35 de-identified clinical specimens using a CLIA/CAP-validated qPCR panel targeting 17 bacteria, 4 fungi, 6 antimicrobial resistance genes, and 1 control gene. Diaper-derived samples demonstrated 100 % concordance in positive and negative predictive values with standard urine specimens. Across all clinical samples, mean cycle threshold (Ct) differences ranged from –2.06 to 3.87 (mean absolute difference = 1.43), with lower variability in diaper samples (SD = 4.02 vs. 4.48) and strong correlation in Ct values ($r = 0.97$). These findings validate the diaper matrix as a clinically robust, non-invasive alternative that maintains diagnostic integrity under simulated transport and storage. This approach enables accurate molecular detection of uropathogens while minimizing invasive procedures, offering immediate applicability for infants, the elderly, and individuals with incontinence—thereby enhancing diagnostic access, accuracy, and antimicrobial stewardship in vulnerable populations.

1. Introduction

The urinary system is essential for maintaining urinary homeostasis [1]. However, this delicate balance is often disrupted by urinary tract infections (UTIs), a widespread health concern affecting nearly half of the global population at least once in their lifetime [2]. Moreover, recurrent UTIs are prevalent, particularly affecting females [3]. Timely diagnosis is crucial, particularly for vulnerable populations, yet traditional urine cultures often fail to detect pathogens, especially following antibiotic use or in the case of hard-to-culture microorganisms [4]. To address these limitations, advanced diagnostics beyond culture-based methods are increasingly being adopted to improve the accuracy and speed of UTI detection [5–8]. A significant focus has been on polymerase chain reaction (qPCR), which offers greater sensitivity and specificity compared to traditional urine culture, particularly for detecting fastidious or low-abundance uropathogens [9–12].

Collecting midstream urine samples is challenging in elderly and pediatric populations, particularly infants and those with incontinence or neurodegenerative conditions like dementia. Standard methods are impractical, and catheterization poses risks of distress and nosocomial infections [13]. Elderly individuals, especially in care facilities where UTI prevalence reaches up to 50 %, face severe risks like urosepsis, hospitalization, and delirium [14,15]. High UTI rates also drive antibiotic overprescription and antimicrobial resistance [16]. Consequently, focus has shifted to evaluating non-invasive methods, such as sodium polyacrylate-based diapers, for UTI diagnostics [17–20].

Although extraction-based diaper techniques are well-established in clinical settings, they are underutilized in molecular diagnostics. While Shvartzman and Nasri [17] reported high sensitivity and specificity with culture-based methods, these methods fall short of the accuracy offered by qPCR [11]. Other approaches, such as colorimetric detection of urinary biomarkers like pH, leukocytes, and nitrites, can indicate the presence of a UTI but lack the specificity to identify the pathogen involved [18]. Point-of-care, diaper-embedded diagnostic tools provide rapid screening [19,20], but their inability to differentiate between uropathogens limits their precision compared to qPCR. In contrast,

* Corresponding author.
E-mail addresses: rcarpenter@uttyler.edu, rec@rlventures.com (R.E. Carpenter).

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Inami and Inoue [21] demonstrated PCR-based detection of Cytomegalovirus (CMV) using urine absorbed by filter paper placed inside baby diapers. Their study effectively isolated CMV from known cases but did not address the viability of using sodium polyacrylate-based diapers for molecular testing.

The present study aims to validate a Clinical Laboratory Improvement Amendments (CLIA) laboratory-developed test (LDT) [22] that uses sodium polyacrylate-based diapers for urine collection, followed by qPCR analysis. This method facilitates non-invasive urine collection for individuals unable to provide midstream samples, such as infants and the elderly. Our findings show that urine collected from diapers remains viable for qPCR analysis with minimal loss of diagnostic sensitivity. This approach offers a promising improvement in UTI diagnostics, enhancing both accuracy and accessibility for challenging patient populations.

2. Materials and methods

This study builds upon previous research involving sodium polyacrylate-based diapers as non-invasive matrices for urine collection in molecular diagnostics from contrived samples [23]. In contrast, the research expands the analysis with a broader dataset of 35 de-identified clinical samples, encompassing both positive and negative cases, to provide enhanced diagnostic insights. Furthermore, this study extends the methodology in several critical ways. It includes statistical analysis demonstrating 100 % positive and negative predictive values (PPV and NPV) between diaper-derived and traditional urine samples, validating the diagnostic concordance. Additionally, it uniquely evaluates the stability of diaper-derived urine samples under simulated transport and storage conditions, a novel aspect not addressed in the earlier work. Finally, the current research contextualizes its findings within clinical workflows by addressing practical considerations such as antimicrobial stewardship and scalability, thereby supporting the broader adoption of this non-invasive diagnostic method. By explicitly building upon the prior dataset [23], this manuscript provides a clinical advancement of the methodology, offering actionable insights for clinical practice. To ensure comprehensive contextualization, we include the methodology of contrived sample testing in this study.

2.1. Optimizing urine extraction from diaper materials

This study aimed to optimize urine extraction from sodium polyacrylate-based diapers to enable accurate pathogen detection through molecular diagnostics. The qPCR method used can identify 22 uropathogens, 6 fungal species, and 18 antimicrobial resistance (AMR) genes (Table 1). The main objective was to validate these diapers as a viable non-invasive urine collection method for patients where conventional collection is impractical. Clinically contrived samples, spiked with known uropathogens, were used to simulate real-world scenarios.

2.2. Samples

Urine samples (1 µL) from clinical specimens received from Advanta Genetics (Tyler, Texas; www.aalabs.com) were inoculated onto Blood Agar Plates (Remel™, TSA with Sheep Blood) and CDC Anaerobic Blood Agar Plates (Remel™) using 1 µL disposable inoculation loops (Thermo Scientific, Blue Disposable Inoculation Loop). The streaking pattern used is illustrated in Fig. 1. Blood agar plates were incubated aerobically at 37°C for 24-48 hours with 5 % CO_2, while CDC plates were incubated anaerobically at 37°C for 24-48 hours using anaerobic gas pouches (BD GasPak™ EZ Anaerobe Gas Generating Pouch System with Indicator).

Following incubation, isolated colonies were resuspended in demineralized water (Thermo Scientific, Sensititre™ Demineralized Water) and vortexed for 10 seconds at maximum speed using a Vortex-Genie 2. The bacterial suspension was standardized to a 0.5 McFarland Standard using a nephelometer (Thermo Scientific, Sensititre Nephelometer). Once standardized, 10 µL of the bacterial suspension was inoculated into 11 mL of Cation-Adjusted Mueller-Hinton Broth with TES (Thermo Scientific, Sensititre™). The inoculated broth was then aliquoted into ID plates (Thermo Scientific, Sensititre™ GPID or GNID) via the Sensititre Aim liquid handling system, based on the results of the initial Gram stain. Additionally, a subset of the broth was streaked onto 1/6th of an agar plate (Fig. 2) to verify the purity of the isolated colonies used for identification via the Sensititre ARIS HiQ system (Thermo Scientific).

Identification numbers were assigned to each organism, and the colonies were selected as spiking candidates. Clinical isolates obtained from Advanta Genetics were chosen to ensure variability, representing a wide spectrum of categories and Gram-staining characteristics (Table 2). This diversity allows for a comprehensive evaluation of the test's diagnostic capabilities across multiple pathogen types.

Table 1
PCR Panel of Uropathogens, Fungal Species, and Antimicrobial Resistance Genes.

Master Mix Solution	FAM	SUN	CY5
1	*Enterococcus faecalis*	*Enterococcus faecium*	*Enterobacter cloacae*
2	*Streptococcus agalactiae*	*Streptococcus pyogenes*	*Klebsiella aerogenes*
3	*Proteus mirabilis*	*Klebsiella pneumoniae*	Vancomycin resistance gene M (VanM)
4	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Morganella morganii*
5	*Candida albicans*	*Klebsiella oxytoca*	*Proteus vulgaris*
6	*Candida tropicalis*	*Candida parapsilosis*	*Aerococcus urinae*
7	New Delhi Metallo-β-lactamase (NDM)	BLANK	*Actinotignum schaalii*
8	RNAseP	*Candida glabrata*	*Escherichia coli*
9	Klebsiella pneumoniae carbapenemase (KPC)	Temoniera β-lactamase (TEM)	*Citrobacter species*
10	Oxacillinase (OXA)	Tetracycline resistance gene M (tetM)	*Acinetobacter baumannii*
11	Aminoglycoside nucleotidyltransferase (ant-1a)	Sulfhydryl variable β-lactamase (SHV)	*Serratia marcescens*
12	Aminoglycoside phosphotransferase (aph3)	Gyrase A (gyrA)	*Prevotella bivia*
13	Quinolone resistance (qnr)	Tetracycline resistance gene B (tetB)	*Staphylococcus saprophyticus*
14	Methicillin resistance gene (MecA)	Vancomycin resistance gene (VanA)	*Bacteroides fragilis*
15	Sulfonamide resistance gene 1 (Sul1)	Dihydrofolate reductase type A1 (DfrA1)	Vancomycin resistance gene B (VanB)
16	*Epidermophyton floccosum*	*Trichophyton rubrum*	Cefotaximase-Munich 1 (CTXM1)

2.3. Diaper spiking procedure

Urine samples (50 mL each) were collected from 7 healthy adult volunteers using a midstream clean-catch method in sterile specimen containers (LabAid™, Sterile Specimen Container with Temperature Strip). These initial samples were not screened for microbial contamination, as the primary goal of this experiment was to validate the feasibility of recovering known, intentionally introduced uropathogens from sodium polyacrylate-based diaper matrices using our Clinical Laboratory Improvement Amendments (CLIA) and College of American Pathologists (CAP) accredited qPCR laboratory-developed test (LDT). Each sample was inoculated with single colonies of pre-characterized

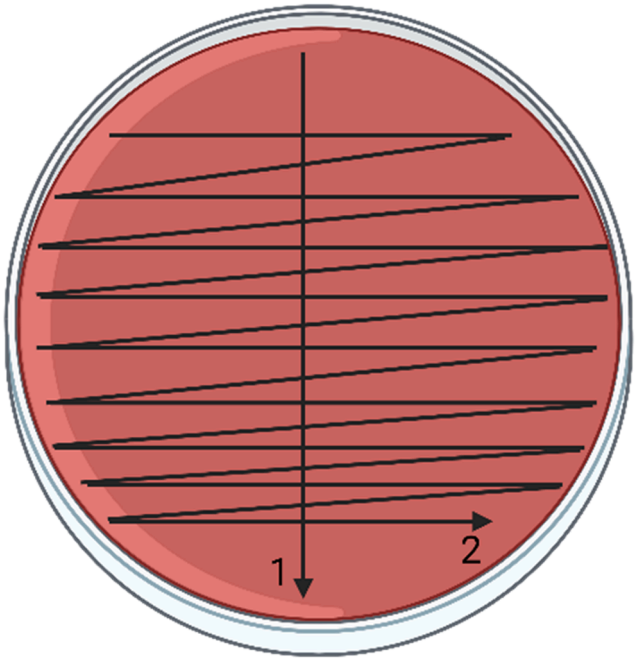

Fig. 1. Streaking pattern on agar plate for isolation of uropathogens. This figure illustrates the streaking pattern used to isolate uropathogenic microorganisms on Blood Agar Plates (BAP) and CDC Anaerobic Blood Agar Plates. The streaking pattern ensures individual colonies are separated for accurate identification and subsequent testing. Plates were incubated under appropriate aerobic or anaerobic conditions to facilitate the growth of targeted organisms.

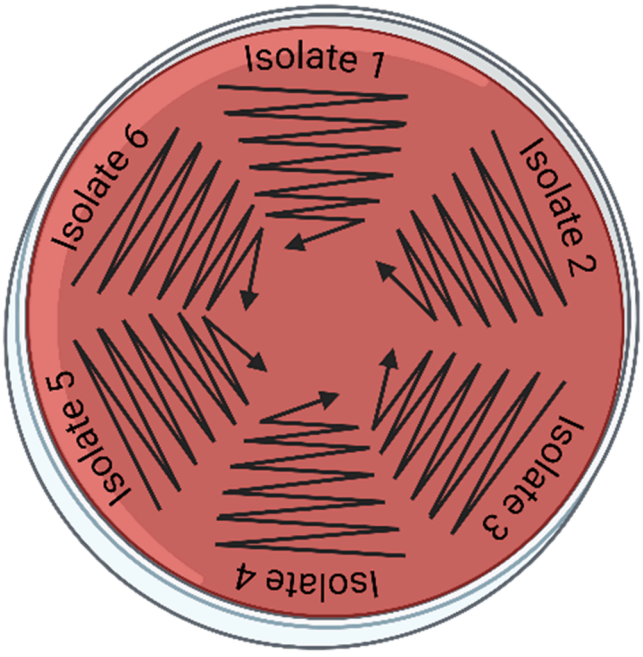

Fig. 2. Purity plate layout for verification of isolated colonies. The plating layout used to confirm the purity of isolated colonies prior to further testing. This step is critical to ensure the reliability of qPCR results and minimize the potential for contamination from non-target microorganisms. The plates were divided into sectors to maximize space and reduce sample cross-contamination.

uropathogens (Table 3), scraped from purity plates using a sterile 1 μL inoculation loop. After inoculation, the urine samples were sealed and vortexed for a minimum of 30 seconds at maximum speed using a Vortex-Genie 2 to ensure homogeneity.

Table 2
Clinical Isolates Used for Microbiological Evaluation, Including Gram Stain, Morphology, and Classification.

Microorganism	Gram	Shape and Arrangement	Category
Enterobacter cloacae	-	Rod-shaped (bacilli)	Enterobacteriaceae family
Enterococcus faecalis	+	Cocci in pairs or chains	*Enterococcus* species; Group D Streptococcus
Enterococcus faecium	+	Cocci in pairs or chains	*Enterococcus* species; Group D Streptococcus
Escherichia coli	-	Rod-shaped (bacilli)	Enterobacteriaceae family
Klebsiella oxytoca	-	Rod-shaped (bacilli)	Enterobacteriaceae family
Klebsiella pneumoniae	-	Rod-shaped (bacilli)	Enterobacteriaceae family
Prevotella bivia	-	Anaerobic rod-shaped (bacilli)	Anaerobic Gram-negative bacilli
Proteus mirabilis	-	Rod-shaped (bacilli)	Enterobacteriaceae family
Pseudomonas aeruginosa	-	Rod-shaped (bacilli)	Non-fermenting Gram-negative bacilli; commonly associated with nosocomial infections
Staphylococcus aureus	+	Cocci in clusters	Gram-positive cocci; *Staphylococcus* species
Streptococcus agalactiae	+	Cocci in chains	Gram-positive cocci; Group B Streptococcus; Beta-hemolytic

Table 3
Uropathogens and Diaper Brands Used in Spiking Procedure for qPCR Analysis.

Urine #	Uropathogens	Diaper Brand
1	*Enterococcus faecalis, Klebsiella oxytoca*	TENA® ProSkin Stretch™
2	*Enterococcus faecium*	FitRight® OptiFit™ Briefs
3	*Klebsiella pneumoniae, Enterobacter cloacae*	Cardinal Health™ Sure Care™ Plus Heavy Absorbency Underwear
4	*Pseudomonas aeruginosa, Staphylococcus aureus (MRSA)*	Prevail® Per-Fit® Daily Underwear
5	*Escherichia coli*	FitRight® Underwear
6	*Proteus mirabilis*	Huggies® OverNites Diapers
7	*Streptococcus agalactiae, Prevotella bivia*	Pampers Baby-Dry

For each spiked urine sample, 25 mL was applied directly to the inner surface of various diaper brands (Table 3), simulating the soiling process. The remaining 25 mL each urine sample was reserved separately for parallel qPCR analysis. The soiled diapers were left at room temperature for 4 hours, followed by refrigeration at 4°C for an additional 24 hours, typical clinical transport and storage delays. The remaining urine samples were similarly stored—4 hours at room temperature followed by 24 hours at refrigerated temperatures—to maintain consistency between diaper-derived and traditional urine sample analyses.

2.4. Diaper wearing cohort

A cohort of 10 volunteers (5 females, 5 males) was recruited and assigned to wear diapers (Equate, Assurance Underwear Maximum Absorbency L/XL) for a minimum of 8 hours without altering their normal daily activities. Each volunteer was provided with a 24-hour urine collection jug (McKesson, Male Urinal, 1 Quart/1000 mL) and instructed to return the full volume of urine from a single elimination. Upon completion of the wear period, the soiled diapers were placed in biohazard bags (Uline, 12 × 15″ Specimen Bags).

Upon receipt, the urine collected from each volunteer was entirely poured onto the internal surface of their corresponding previously worn diaper, accurately simulating the real-world scenario of diaper-based urine collection. No measures were taken to prevent contamination

from normal skin or genital mucosa microflora, as such contamination is inevitable in actual clinical practice. This intentional inclusion ensured the method's robustness under realistic conditions.

Diaper samples were held at room temperature for 4 hours, followed by refrigeration at 4°C for 24 hours, consistent with conditions used in the spiked diaper studies. To evaluate potential contamination effects, each diaper-derived sample was processed and analyzed twice: initially without spiking, confirming that natural microflora did not cause false-positive results, and subsequently spiked with positive QC material at the lower limit of detection (LLoD) to verify that diaper material did not impair sensitivity compared to a control sample (TE buffer spiked at the same concentration).

2.5. Urine recovery procedure

Diapers were placed on a sterilized stainless-steel surface that had been treated with 10 % bleach, allowed to dry, and then wiped with 98 % ethanol. The internal surface of each diaper was exposed by carefully splaying it open. A sterilized razor blade (WorkPro 61 mm Boxcutter) treated with the same sterilization process (10 % bleach, drying, 98 % ethanol) was used to make an incision down the center of the diaper lining, ensuring the fabric was lifted cleanly away from the diaper's absorbent batting. The incision was extended using sterilized stainless-steel tongs (Eddeas® stainless steel cooking tongs), providing easy access to the soiled diaper's batting.

The soiled batting was transferred into a sterile specimen container (LabAid™, Sterile Specimen Container with Temperature Strip) until it reached three-quarters of the container's capacity. Next, 2.5 grams of calcium chloride (Thermo Fisher, Calcium Chloride, Anhydrous 93 %) was added to the container. This is because sodium polyacrylate (NaPA), the diaper's superabsorbent polymer, retains fluid via ionic interactions between its negatively charged carboxylate groups and positively charged sodium ions (Na+). Calcium chloride ($CaCl_2$) is added to facilitate an ionic exchange reaction with NaPA:

$$2Na(PA) + CaCl2 \rightarrow Ca(PA)2 + 2NaCl \quad (1)$$

Here, calcium ions (Ca^{2+}) form cross-links between carboxylate groups in the polymer, effectively shrinking the polymer matrix and releasing the absorbed liquid. Thus, $CaCl_2$, or another similarly charged cation, is essential for efficiently recovering urine from the sodium polyacrylate crystals for subsequent molecular testing. The container was sealed and shaken vigorously to disperse the calcium chloride evenly, causing a slight increase in temperature detectable by hand.

After the $CaCl_2$ was mixed thoroughly, the container was unsealed. The contents were compressed using a stainless-steel cocktail muddler (TrippleLife, 8″ Cocktail Muddler) to release the urine absorbed in the sodium polyacrylate crystals. The liberated urine was then transferred using a 7 mL polyethylene transfer pipette (Globe Scientific) into a 15 mL conical tube (Axygen, SCT-15mL-500). This recovered urine was subsequently processed according to standard nucleic acid extraction protocols [6,24]. For a visual summary of this workflow, refer to Fig. 3.

2.6. Total nucleic acid extraction

For each urine sample, 600 µL was transferred into a 1.5 mL conical tube (Eppendorf, 1.5 mL FlexTubes, natural) pre-loaded with RNase-free zirconium oxide beads (Nextadvance, Zirconium Oxide Beads, RNase-Free, 0.5 mm diameter, 4 mL) and 20 µL of Proteinase K (Invitrogen, Proteinase K, 20 mg/mL). Lysis was performed using the QIAGEN TissueLyser II at 30 Hz for 5 minutes to ensure thorough disruption of cells and efficient release of nucleic acids. Following lysis, 200 µL of the lysate from each sample was transferred to a 96-well deep-well plate (Roche, MagNA Pure 96 Deep-Well Plate) for nucleic acid extraction using the Roche MagNA Pure 96 system. The extraction process was carried out with the Pathogen Universal 3.0 protocol, utilizing commercially available reagents from the Roche MagNA Pure 96 DNA and Viral Nucleic Acid Small Volume Kit (Roche, Basel, Switzerland). Each sample was eluted in a final volume of 100 µL, which was then used for subsequent qPCR analysis to assess the presence and quantity of specific nucleic acids.

2.7. Diaper matrix effects

Total nucleic acids extracted from diapers worn by the 10 volunteers were processed in duplicate and transferred into 1.5 mL conical tubes (Eppendorf, 1.5 mL FlexTubes, natural). One aliquot from each sample was evaluated without any modifications, while the second aliquot was spiked with synthetic double-stranded DNA (dsDNA) controls (1000 c/uL PC; www.scienetix.com) at the LLoD of the assay (10 c/uL). The purpose of spiking the second aliquot was to ensure that no PCR inhibitors were present in the sample preparation or extraction processes [25], which could otherwise reduce the assay's sensitivity at the LLoD [26]. In parallel, an aliquot of Tris-EDTA buffer was also spiked with synthetic dsDNA at the LLoD to serve as the reference or true value against which all diaper-derived sample results were compared. For quality control, unspiked aliquots were tested to confirm that no pathogenic targets were detected in any of the 10 diaper-derived samples, verifying the absence of cross-reactivity due to microflora contamination from prolonged (8-hour) diaper wear. This step was crucial to rule out false-positive results that could arise from environmental contamination during wear [27].

2.8. De-identified clinical samples and diaper batting comparison

A total of 35 de-identified and previously characterized clinical samples, sourced from Advanta Genetics (www.aalabs.com), were analyzed using a CLIA/CAP-validated qPCR panel specifically designed for the detection of UTI pathogens. Following the initial qPCR evaluation, 2 mL of each sample were transferred into sample collection tubes (Sarstedt, 10 mL Tubes) containing fresh, dry diaper batting material. The batting, sourced from new, unused diapers (Equate Assurance Underwear, Maximum Absorbency, L/XL), occupied approximately one-third of the tube's volume. These prepared samples were stored under refrigeration overnight to assess the stability and compatibility of the storage medium. Subsequent qPCR analysis using the same CLIA/CAP-validated UTI panel was performed to directly compare the results of the diaper-based storage approach to the original characterization, allowing for a comparative analysis of the method's impact on test integrity and accuracy.

2.9. Pathogen detection

A total of 34 samples were processed through the total nucleic acid extraction procedure. This included seven urine samples spiked with cultured uropathogens, seven corresponding diaper samples spiked with half the volume of their paired urine samples, and 10 urine samples collected from worn diapers, each representing the volume of a complete elimination. These 10 urine samples were processed in duplicate and divided into two groups: spiked and unspiked, to assess the impact of the diaper matrix with respect to qPCR sensitivity [26]. All samples, including spiked and unspiked groups, were analyzed for the presence of 22 uropathogens, 6 fungal species, and 18 antimicrobial resistance (AMR) genes (Table 3). Commercially available pre-designed PCR reaction mixtures (www.scienetix.com, Tyler, TX, USA) were used for all assays. The total nucleic acid extracted from each sample (2.5 µL) was added to each reaction mixture, totaling 80 µL of extracted material per sample across multiple reactions.

The reaction mixtures (RM1–RM16) were added to a 384-well plate (Roche, LightCycler® 480 Multiwell Plate 384, White) as per the layout described in Fig. 4. Each plate included both a positive amplification control and a negative amplification control to ensure assay reliability

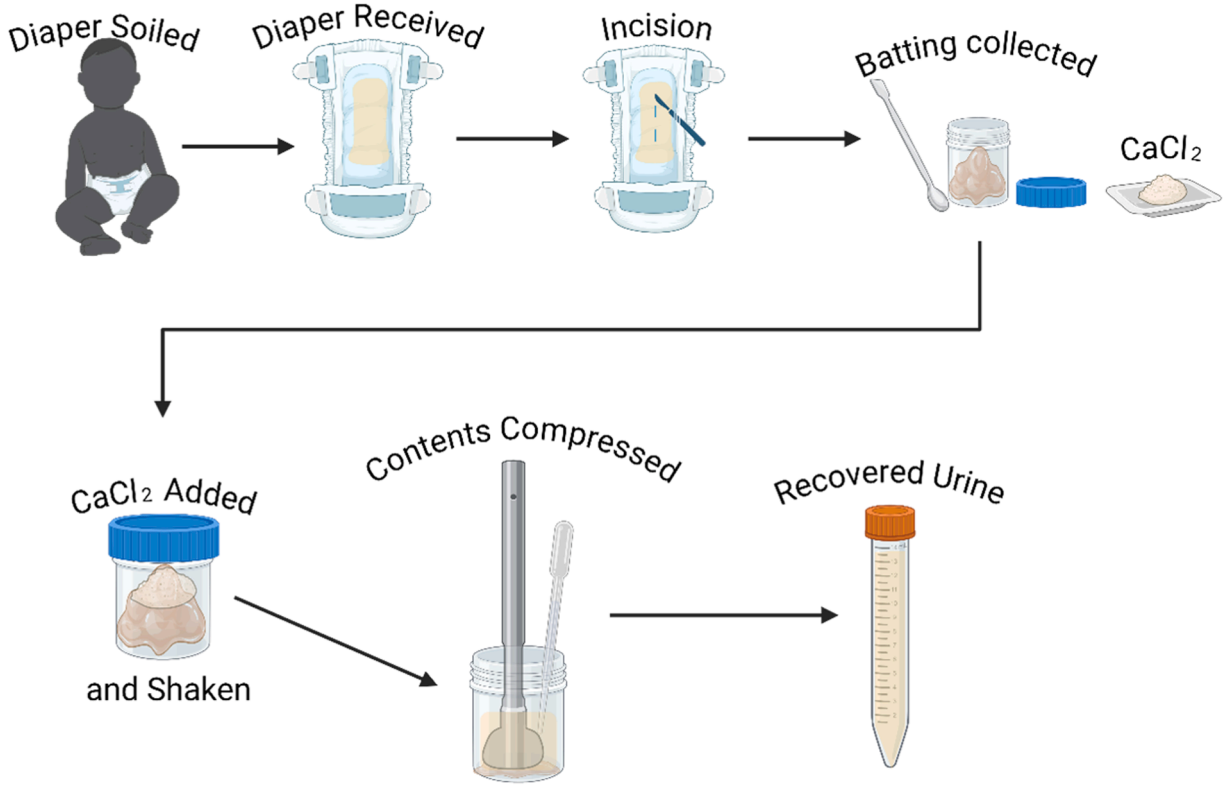

Fig. 3. Workflow for recovering urine from sodium polyacrylate-based diapers for molecular diagnostics. This visual summarizes the step-by-step process for urine recovery from sodium polyacrylate-based diapers. It includes diaper disassembly, extraction of urine from absorbent material, and preparation of the recovered urine for nucleic acid extraction. Key tools and reagents are highlighted to ensure reproducibility of the workflow.

Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12	Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Positive Controls	Negative Controls
RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1
RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2
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RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15
RM16	RM16	RM16	RM16	RM16	RM16	RM16	RM16	RM16	RM16	RM16	RM16	RM16	RM16	RM16	RM16	RM16	RM16	RM16	RM16	RM16	RM16	RM16	RM16

Fig. 4. Layout of PCR reaction mixtures in 384-well plate. Each well is designated for specific targets, including bacterial species, fungal pathogens, antimicrobial resistance genes, and controls. This layout ensures efficient and simultaneous detection of multiple targets in a high-throughput qPCR format. Each column is dedicated to a single sample and columns 23 and 24 are reserved for positive and negative amplification controls. Positive controls confirm assay sensitivity, while negative controls verify the absence of contamination or false-positive amplification. The control placement ensures assay reliability and data integrity.

[28,29].

3. Results

3.1. Urine and diapers spiked with uropathogens

Urine and diaper pairs spiked with uropathogens were analyzed via qPCR following nucleic acid extraction using a laboratory-developed test (LDT) validated by Advanta Genetics, a CLIA-certified, CAP-accredited laboratory (see Table 1 for assay). All spiked uropathogens were detected in both urine and diaper-recovered samples (Table 4), demonstrating reliability of the extraction and qPCR protocols.

Table 4
Comparative qPCR Detection of Uropathogens in Spiked Urine and Diaper-Derived Samples.

Sample #	Target	Diaper Ct	Urine Ct	Δ Ct	% Diff
1	*Enterococcus faecalis*	20.23	20.67	0.44	2.13 %
1	*Klebsiella oxytoca*	24.31	22.40	−1.91	−8.53 %
2	*Enterococcus faecium*	31.40	29.98	−1.42	−4.74 %
3	*Enterobacter cloacae*	26.77	25.32	−1.45	−5.73 %
3	*Klebsiella pneumoniae*	20.64	17.57	−3.07	−17.47 %
3	Sulfhydryl Variable β-lactamase (SHV)	20.32	18.24	−2.08	−11.40 %
4	*Pseudomonas aeruginosa*	22.79	21.94	−0.85	−3.87 %
4	*Staphylococcus aureus*	22.51	20.61	−1.90	−9.22 %
4	Methicillin resistance gene (MecA)	23.39	21.49	−1.90	−8.84 %
5	*Escherichia coli*	22.59	20.45	−2.14	−10.46 %
5	Quinolone resistance gene (qnr)	22.64	20.26	−2.38	−11.75 %
6	*Proteus mirabilis*	23.27	21.94	−1.33	−6.06 %
7	*Streptococcus agalactiae*	34.53	34.51	−0.02	−0.06 %
7	*Prevotella bivia*	32.78	29.68	−3.10	−10.44 %

3.2. Matrix effects of diapers

Diapers worn for at least 8 hours, then spiked with a single urine volume, were processed through the nucleic acid extraction protocol in duplicate. One aliquot from each sample underwent qPCR to determine if microflora accumulated during wear would cause false positives. The results showed no universal detection of pathogens across samples, and no pathogens were found in the volunteer-derived samples, confirming that the diaper microflora did not significantly cross-react after 8 hours of wear, eliminating concerns about false positives in qPCR analysis.

The second aliquot was spiked at the lower limit of detection (LLoD, 10 c/uL) for all targets and analyzed. A Tris-EDTA buffer control spiked at the same LLoD served as a reference. All spiked targets in the diaper samples successfully amplified, demonstrating that detection at the LLoD was achievable. Comparing Ct values from spiked diaper samples to the Tris-EDTA buffer control, all targets fell within ±3.33 cycles of the control, confirming that diaper material did not significantly affect the sensitivity or accuracy of detection at the LLoD. Although slight variability in Ct values was observed, positive amplification of all targets confirmed the integrity of the qPCR assay in the presence of diaper material.

3.4. Comparative qPCR detection of uropathogens

Fig. 5 shows the mean Ct value for diaper-derived samples was 24.87 (±4.70), while the mean Ct for urine samples was 23.22 (±4.90). The average ΔCt between diaper and urine samples was −1.65, indicating that Ct values from diaper samples were, on average, 1.65 cycles higher, which suggests a minor reduction in sensitivity associated with urine recovery from diaper matrices. The mean percentage difference between the two sample types was −7.60 %, indicating a modest decrease in detection sensitivity.

A high Pearson correlation coefficient ($r = 0.979$) was observed between Ct values of diaper-derived and urine samples, demonstrating strong concordance in detection outcomes between the two matrices. The Ct values for diaper samples ranged from 20.23 to 34.53, while those for urine samples ranged from 17.57 to 34.51, indicating comparable variability across the sample types. Notably, no sample exhibited a ΔCt greater than 3.33 cycles, which corresponds to a 10-fold dilution, accentuating that significant sensitivity loss was not observed in any sample.

The median Ct values were 23.03 for diaper samples and 21.72 for urine samples, with a mean ΔCt of −1.65 cycles and a 95 % confidence interval of [−2.23, −1.07]. Cohen's d was 0.34, indicating a small to medium effect size. No significant outliers were found based on ΔCt

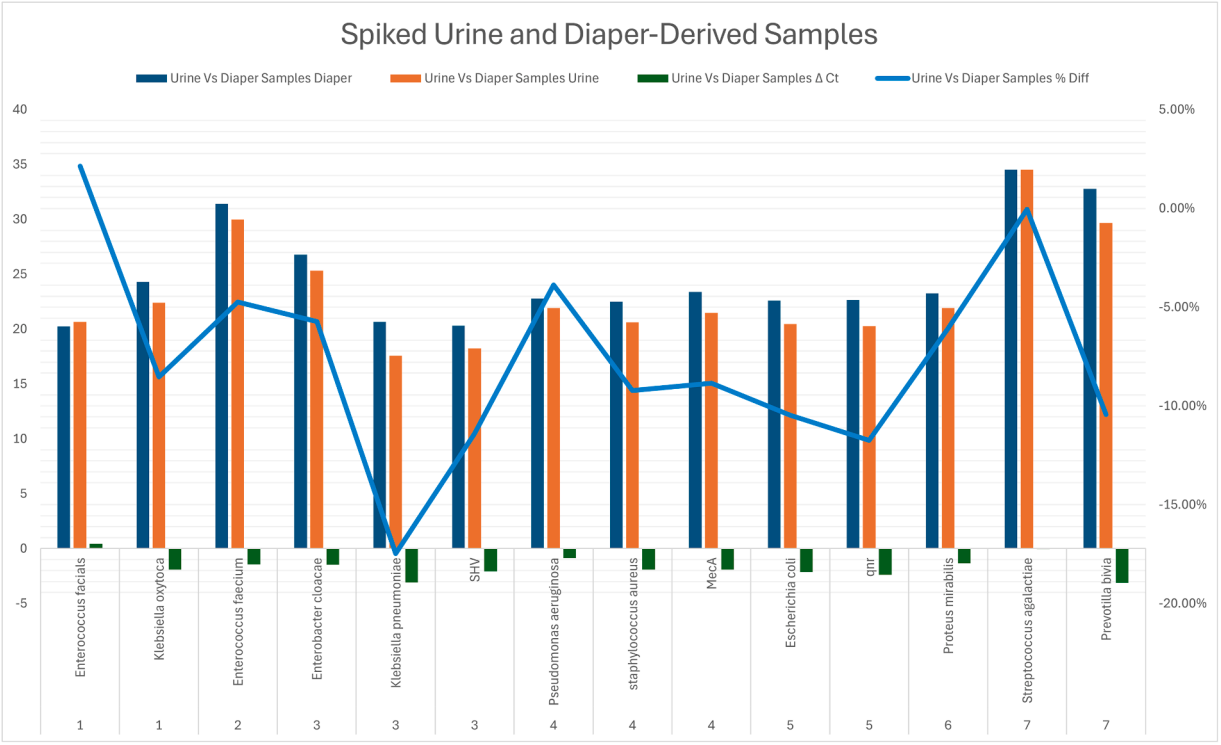

Fig. 5. Graphical comparison of qPCR detection results between spiked urine and diaper-derived samples. This graph highlights cycle threshold (Ct) value ranges, mean differences, and statistical correlation (Pearson $r = 0.979$), emphasizing the diagnostic equivalence of the two sample matrices despite minor sensitivity variations.

values. Despite minor sensitivity reduction in diaper-derived samples, the strong correlation between diaper and urine Ct values ($r = 0.979$) and the small effect size suggest minimal interference from the diaper matrix in detecting uropathogens. Given that a 3.33 Ct cycle difference equates to a 10-fold dilution, this slight loss in sensitivity could be mitigated by sample concentration.[1]

3.5. Evaluating diaper matrices for diagnostic concordance with clinical urine samples

Both clinically positive samples ($n = 22$) and clinically negative samples ($n = 13$) sourced from Advanta Genetics (www.aalabs.com) were spiked onto diaper batting (Equate, Assurance Underwear Maximum Absorbency L/XL) and compared to their corresponding urine counterparts to evaluate the reliability and diagnostic consistency of the diaper-based sample recovery method (Table 5). These spiked samples underwent rigorous assessment for concordance with unaltered, previously characterized clinical specimens. The analysis encompassed 17 bacterial targets, 4 fungal targets, 6 antimicrobial resistance (AMR) targets, and 1 control target (RNAseP), representing a comprehensive array of clinically relevant pathogens and resistance markers encountered in diagnostic practice. Across all 35 samples, the results demonstrated 100 % PPV and NPV concordance in target detection, with all targets remaining detectable following 24 hours of refrigerated exposure to the diaper batting. Mean Ct differences between the diaper and urine matrices ranged from −2.06 to 3.87, with a mean absolute Ct difference of 1.43 across all targets. Notably, critical targets such as Escherichia coli and Staphylococcus aureus exhibited mean Ct differences of −0.36 and 2.93, respectively, emphasizing the diaper matrix's comparable sensitivity. The diaper matrix maintained consistent diagnostic integrity under simulated transport and storage conditions, supporting its viability as an alternative collection matrix.

Statistical analysis reinforces the suitability of the diaper matrix as a viable alternative to the urine matrix for clinical diagnostics. The correlation coefficient ($r = 0.97$) indicates a strong positive relationship between the Ct values of the two matrices, highlighting their comparable performance in target detection. Additionally, the diaper matrix demonstrates slightly lower variability, with a standard deviation (SD = 4.02) compared to the urine matrix (SD = 4.48), and a smaller coefficient of variation (CV% = 13.41 % vs. 15.12 %), indicating more consistent Ct values relative to the mean. While a paired t-test reveals a statistically significant difference in Ct values between the matrices (t-statistic = 2.85, p-value = 0.0068), these differences likely stem from matrix-specific properties or handling factors, which can be addressed through calibration protocols or interpretive adjustments to ensure diagnostic accuracy.

Together, these findings substantiate the diaper matrix as a reliable and consistent alternative, offering comparable detection capabilities. The study also highlights practical advantages of the diaper matrix, such as its ease of sample collection, particularly in pediatric and mobility-restricted populations, and its potential for non-invasive diagnostics. These results position the diaper matrix as a viable tool for expanding accessibility and flexibility in clinical diagnostic practices.

4. Limitations

While this study demonstrated the feasibility of using sodium polyacrylate-based diapers for molecular UTI diagnostics, several

[1] To address this, 6 mL of diaper-recovered urine can be centrifuged to form a pellet. The pellet may then be resuspended in 600 μL of either the original supernatant or Tris-EDTA buffer (Fisher Bioreagents, 1x Solution, pH 8.0) to concentrate the sample. The concentrated urine would undergo lysis as described in prior protocols, compensating for the sensitivity loss observed in the cycle threshold (Ct) values and improving detection accuracy.

Table 5
Comparative Analysis of Urine and Diaper Matrices for Clinically Positive and Negative Samples.

qPCR Target	TP	TN	FP	FN	Mean Diaper CT	Mean Urine CT	Mean $\Delta Ct^{(Diaper-Urine)}$
Acinetobacter baumannii	0	35	0	0	NA	NA	NA
Actinotignum schaalii	2	33	0	0	28.09	27.13	0.96
Aerococcus urinae	2	33	0	0	29.78	31.19	−1.41
Bacteroides fragilis	3	32	0	0	27.28	28.27	−0.99
Candida albicans	1	34	0	0	26.46	24.29	2.17
Candida auris	0	35	0	0	NA	NA	NA
Candida glabrata	3	32	0	0	27.28	26.66	0.62
Candida parapsilosis	0	35	0	0	NA	NA	NA
Candida tropicalis	1	34	0	0	23.50	25.56	−2.06
Citrobacter freundii/braakii/koseri	1	34	0	0	17.59	15.66	1.93
Enterobacter cloacae	7	28	0	0	30.21	27.57	2.64
Enterococcus faecalis	10	25	0	0	25.68	23.74	1.94
Enterococcus faecium	1	34	0	0	31.96	33.26	−1.3
Epidermophyton floccosum	0	35	0	0	NA	NA	NA
Escherichia coli	12	23	0	0	23.05	23.41	−0.36
Klebsiella aerogenes	2	33	0	0	21.53	19.16	2.37
Klebsiella oxytoca	3	32	0	0	24.43	25.03	−0.6
Klebsiella pneumoniae	5	30	0	0	25.53	22.78	2.75
Morganella morganii	1	34	0	0	18.80	14.93	3.87
Prevotella bivia	3	32	0	0	32.47	31.32	1.15
Proteus mirabilis	0	35	0	0	NA	NA	NA
Proteus vulgaris	0	35	0	0	NA	NA	NA
Pseudomonas aeruginosa	1	34	0	0	24.84	21.54	3.3
Serratia marcescens	0	35	0	0	NA	NA	NA
Staphylococcus aureus	2	33	0	0	23.67	20.74	2.93
Staphylococcus haemolyticus	3	32	0	0	30.69	29.18	1.51
Staphylococcus saprophyticus	1	34	0	0	23.62	21.94	1.68
Streptococcus agalactiae (Group-B)	3	32	0	0	24.11	22.99	1.12
Streptococcus pyogenes (Group-A)	0	35	0	0	NA	NA	NA
Trichophyton rubrum	0	35	0	0	NA	NA	NA
Beta Lactamase Resistance (SHV)	4	31	0	0	23.77	21.32	2.45
Beta Lactamase Resistance (TEM)	5	30	0	0	23.62	20.85	2.77
Beta Lactamase Resistance (CTX-M-Grp1)	0	35	0	0	NA	NA	NA
Carbapenem Resistance (NDM)	0	35	0	0	NA	NA	NA
Carbapenem Resistance (OXA-48)	0	35	0	0	NA	NA	NA
Fluoroquinolone Resistance (gyrA)	0	35	0	0	NA	NA	NA
Fluoroquinolone Resistance (qnrAS)	0	35	0	0	NA	NA	NA

(continued on next page)

Table 5 (*continued*)

qPCR Target	TP	TN	FP	FN	Mean Diaper CT	Mean Urine CT	Mean $\Delta Ct^{(Diaper-Urine)}$
Methicillin Resistance (MecA)	4	31	0	0	30.77	29.87	0.90
Sulfonamide Resistance (Sul1)	5	30	0	0	32.42	33.69	−1.27
Tetracycline Resistance (TetB)	0	35	0	0	NA	NA	NA
Tetracycline Resistance (TetM)	14	21	0	0	29.99	29.53	0.46
Trimethoprim Resistance (DfrA1)	1	34	0	0	22.00	23.53	−1.53
Vancomycin Resistance (VanA)	0	35	0	0	NA	NA	NA
Vancomycin Resistance (VanB)	0	35	0	0	NA	NA	NA
Vancomycin Resistance (VanM)	0	35	0	0	NA	NA	NA
RNAseP	13	22	0	0	30.92	30.11	0.81

Note: NA = no positive clinical sample.

limitations should be noted. First, diaper materials may introduce inhibitors or reduce sample integrity, leading to increased Ct values and slightly reduced sensitivity compared to clean-catch urine, particularly at low pathogen concentrations [30]. Although sample concentration can mitigate this, further studies are needed to ensure consistency across different diaper brands. Second, prolonged diaper wear may increase environmental contamination risk, even though no false positives were observed [9]. This should be further investigated under varied clinical conditions. Third, variability in urine recovery from soiled diapers may lead to inconsistent sample concentrations, affecting diagnostic accuracy [31]. Concentration steps, while effective, add complexity to routine workflows. Fourth, the efficacy across a broader range of pathogens, including polymicrobial and fastidious organisms, still needs validation [32]. Last, although no significant qPCR inhibition was observed, potential inhibitors from diaper materials or urine substances must be further evaluated across a wider range of diapers and patient populations [33]. Finally, while duplicate testing aligns with clinical laboratory validation guidelines (CLSI EP05-A3) and is adequate for initial method verification, further comprehensive validation incorporating triplicate or higher replicates would provide enhanced statistical rigor and reproducibility assurance.

5. Clinical implications

A validated method for non-invasive urine collection using sodium polyacrylate-based diapers offers several important clinical implications: enabling non-invasive sample collection for difficult-to-serve populations, supporting rapid and accurate pathogen detection, improving antimicrobial stewardship, enhancing diagnostic specificity, mitigating MDRO risks, and extending the reach of advanced diagnostics. Together, these benefits highlight the potential of this approach in improving patient care, particularly for populations at high risk of inappropriate antibiotic treatment and infection-related complications.

5.1. Non-invasive urine collection

The first clinical implication is the non-invasive nature of the approach, which is particularly beneficial for populations where midstream collection is impractical. This includes elderly patients with advanced dementia or young children who are not toilet-trained. For elderly residents with cognitive impairments, such as those in nursing homes, assessing suspected UTIs is challenging due to communication barriers and the high risk of antimicrobial resistance [34]. A non-invasive and efficient urine collection method, like the diaper-based approach, allows for consistent and reliable urine sampling without the need for patient cooperation, reducing the challenges and risks of standard invasive methods. This can help mitigate issues associated with UTI overdiagnosis, inappropriate treatment, and antibiotic misuse, particularly for vulnerable populations who cannot adequately communicate symptoms.

5.2. Accurate PCR-based uropathogen detection

The second clinical implication is the enhanced diagnostic accuracy offered by PCR-based detection of uropathogens, which this method facilitates. This approach supports faster, more precise identification of causative pathogens, thereby allowing for targeted antibiotic use and contributing to the reduction of antimicrobial resistance—a key concern in nursing home environments [35]. By avoiding empirical treatment with broad-spectrum antibiotics, healthcare providers can mitigate the development of multidrug-resistant organisms (MDROs). Moreover, the method eliminates the need for invasive catheterization, reducing patient discomfort and the associated risks of secondary infections. The use of PCR, in contrast to traditional culture methods, also provides advantages in diagnosing infections where culture results might be negative or ambiguous, such as when patients are already on antibiotics, when multiple pathogens are present, or when organisms are atypical [36]. This increased sensitivity supports accurate diagnosis, even in challenging circumstances, thereby enhancing clinical decision-making and improving patient outcomes.

5.3. Timely results and their impact on antimicrobial stewardship

The third implication relates to the speed of results. The molecular diagnostic capabilities of this method, such as qPCR, provide results notably faster than traditional urine cultures, which can take between 3 and 12 days depending on the nature of the results (e.g., negative versus positive or contaminated cultures) [11]. Rapid detection facilitates timely clinical decisions, allowing for the prompt initiation, adjustment, or discontinuation of antibiotic therapy as needed. In long-term care facilities, where delays in diagnostic results often lead to the overuse of antibiotics [37], faster diagnostics can directly contribute to more effective antimicrobial stewardship. Studies have shown that while culture results often suggest changes to antibiotic therapy, these changes are implemented in only a minority of cases, primarily due to delays in culture reporting [38]. By reducing these delays, the validated diaper urine extraction method promotes better alignment between clinical practice and evidence-based antimicrobial use, thereby addressing both patient care needs and broader public health concerns related to antimicrobial resistance.

5.4. Enhanced diagnostic reliability and specificity

The fourth clinical implication is the improved diagnostic reliability and specificity of PCR-based testing in distinguishing uropathogenic infections from contamination. By combining non-invasive urine collection with PCR, and potentially supplementing it with embedded nitrite and leukocyte esterase testing strips, this method provides a comprehensive diagnostic tool. Although not considered in this study, the use of diaper-embedded test strips to detect nitrite and leukocyte esterase may further enhance the novelty of this diaper-based technique by preemptible differentiation of true pathogens from contaminants, offering a practical, non-invasive, and robust solution for more comprehensive workflow toward accurate UTI diagnosis [39].

Although there is no universally absolute quantitative criterion, a

commonly accepted threshold for diagnosing UTIs is the detection of >10^5 colony-forming units (CFU) of a single organism per milliliter of urine, which can be applied to PCR quantification as well [40]. In pediatric patients undergoing bladder catheterization, a lower threshold of 10,000 CFU/mL optimally balances sensitivity and specificity [41]. PCR, with its higher sensitivity, provides a diagnostic advantage, especially in situations where traditional cultures may yield negative or inconclusive results. This is often the case for urine samples from patients already on antibiotics, samples containing organisms other than E. coli or Proteus, or from male patients where culture growth may be minimal or absent.

This is particularly crucial for vulnerable populations, such as children undergoing bladder catheterization, where balancing risks and benefits of invasive interventions while ensuring accurate detection of infections is essential. Overall, the enhanced sensitivity and specificity of PCR-based diagnostics improve the identification of uropathogens, ensuring that treatment decisions are based on more precise and reliable data, thereby improving patient outcomes.

5.5. *Implications for mitigating multidrug-resistant organisms*

The fifth clinical implication is the potential impact of this method on mitigating the development and spread of multidrug-resistant organisms (MDROs) in healthcare facilities. Antimicrobial exposure is a significant driver of MDRO colonization, especially in long-term care environments, where inappropriate empirical antibiotic treatment is common [42,43]. The use of timely PCR diagnostics can reduce inappropriate antibiotic exposure, thereby decreasing the risk of MDRO development. In elder care facilities, where the prevalence of MDROs among residents, especially those with advanced dementia, is disproportionately high, a reliable diagnostic method that limits unnecessary antibiotic use can play a crucial role in curbing the spread of resistant pathogens [37]. This has significant public health implications, given the frequent transfer of nursing home residents to hospitals, potentially introducing MDROs into broader healthcare systems [44]. By facilitating precise organism identification and reducing empirical antibiotic use, this diaper extraction method supports efforts to maintain antimicrobial stewardship and improve infection control across healthcare settings.

5.6. *Scalability and practicality in varied healthcare settings*

Finally, the simplicity of the urine extraction method using sodium polyacrylate-based diapers makes it suitable for a broad range of healthcare environments, from large clinical laboratories to smaller, point-of-care settings. Its non-invasiveness and ease of use are particularly advantageous in under-resourced healthcare settings where traditional sample collection methods may be logistically challenging. The scalability of this technique enables broader access to advanced diagnostic capabilities, potentially reducing disparities in healthcare quality between different settings. By providing a reliable and sensitive diagnostic option that is easy to implement, this method can enhance diagnostic capabilities in locations where conventional methods are not feasible, ensuring that vulnerable populations—such as those in long-term care facilities or remote regions—receive timely and appropriate care.

6. Conclusion

This study presents a novel, non-invasive method for UTI diagnostics using sodium polyacrylate-based diapers for urine collection in patients unable to provide clean-catch samples. The optimized extraction technique preserved pathogen detection integrity via qPCR, with minimal matrix effects, even after prolonged wear. These results validate the use of diaper-recovered urine in molecular assays, offering an effective alternative for UTI testing in vulnerable populations such as infants, the elderly, and those with incontinence. This innovative approach not only ensures sample viability and qPCR sensitivity but also overcomes collection barriers, enhancing both patient compliance and diagnostic accuracy—offering a scalable, non-invasive solution that combines reliable sample collection with high-sensitivity molecular testing.

Ethics statement

This study did not require Institutional Review Board approval because it did not involve any human subjects directly. The urine samples used in this research were collected from healthy adult volunteers under minimal risk, non-invasive conditions. The study aimed to validate laboratory methodologies using spiked clinical contrived samples. No personally identifiable information was collected, and the samples were processed in accordance with established CLIA laboratory protocols. This research aligns with the guidelines for exemption from human subjects' research oversight as outlined by regulatory bodies, including the U.S. Department of Health and Human Services (45 CFR 46).

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CRediT authorship contribution statement

Tyler Vine: Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Rob E. Carpenter:** Writing – review & editing, Writing – original draft, Resources, Project administration. **Debbie Bridges:** Writing – review & editing, Methodology.

Declaration of competing interest

Rob E. Carpenter and Tyler Vine are employees of Advanta Genetics, LLC This relationship has been disclosed in accordance with journal guidelines, and all efforts have been made to ensure the objectivity and integrity of the research presented in this study. The remaining authors declare no competing interests.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.diagmicrobio.2025.116939.

References

[1] Behzadi P, Kim CH, Pawlak EA, Algammal A. Editorial: the innate and adaptive immune system in human urinary system. Front Immunol 2023;14:1294869. https://doi.org/10.3389/fimmu.2023.1294869.
[2] Shaikh N, Lee S, Krumbeck JA, Kurs-Lasky M. Support for the use of a new cutoff to define a positive urine culture in young children. Pediatrics 2023;152:061931. https://doi.org/10.1542/peds.2023-061931.
[3] Behzadi P, García-Perdomo HA, Autrán Gómez AM, Pinheiro M, Sarshar M. Uropathogens, urinary tract infections, the host-pathogen interactions and treatment. Front Microbiol 2023;14:1183236. https://doi.org/10.3389/fmicb.2023.1183236.
[4] Price TK, Dune T, Hilt EE, Thomas-White KJ, Kliethermes S, Brincat C. The clinical urine culture: enhanced techniques improve detection of clinically relevant microorganisms. J Clin Microbiol 2016;54:1216–22. https://doi.org/10.1128/jcm.00044-16.
[5] He S, Liu H, Hu X, Zhao J, Liang J, Zhang X, et al. Exploring the clinical and diagnostic value of metagenomic next-generation sequencing for urinary tract infection: a systematic review and meta-analysis. BMC Infect Dis 2024;24:1000. https://doi.org/10.1186/s12879-024-09914-9.
[6] Almas S, Carpenter RE, Rowan C, Tamrakar VK, Bishop J, Sharma R. Advantage of precision metagenomics for urinary tract infection diagnostics. Front Cell Infect Microbiol 2023;13:1221289. https://doi.org/10.3389/fcimb.2023.1221289.
[7] Carpenter RE. Advancing clinical microbiology: applications and future of next-generation sequencing. SAR J Pathol Microbiol 2024;5:107–15. https://doi.org/10.36346/sarjpm.2024.v05i03.00X.
[8] Yang S, Kozyreva VK, Timme RE, Hemarajata P. Integration of NGS in clinical and public health microbiology workflows: applications, compliance, quality

considerations. Front Public Health 2024;12:1357098. https://doi.org/10.3389/fpubh.2024.1357098.
[9] Davenport M, Mach KE, Shortliffe LMD, Banaei N, Wang TH, Liao JC. New and developing diagnostic technologies for urinary tract infections. Nat Rev Urol 2017;14:296–310. https://doi.org/10.1038/nrurol.2017.20.
[10] Szlachta-McGinn A, Douglass KM, Chung UYR, Jackson NJ, Nickel JC, Ackerman AL. Molecular diagnostic methods versus conventional urine culture for diagnosis and treatment of urinary tract infection: a systematic review and meta-analysis. Eur Urol Open Sci 2022;44:113–24. https://doi.org/10.1016/j.euros.2022.08.009.
[11] Wojno KJ, Baunoch D, Luke N, Opel M, Korman H, Kelly C. Multiplex PCR based urinary tract infection (UTI) analysis compared to traditional urine culture in identifying significant pathogens in symptomatic patients. Urology 2020;136:119–26. https://doi.org/10.1016/j.urology.2019.10.018.
[12] Carpenter RE. Beyond standard urine culture: advanced molecular testing for urinary tract infections. SAR J Med 2024;5:80–6. https://doi.org/10.36346/sarjm.2024.v05i03.003.
[13] Lo E, Nicolle LE, Coffin SE, Gould CV, Maragakis LL, Meddings J, et al. Strategies to prevent catheter-associated urinary tract infections in acute care hospitals: 2014 update. Infect Control Hosp Epidemiol 2014;35:464–79. https://doi.org/10.1086/675718.
[14] Cleveland Clinic. The truth about UTIs in older adults. Cleveland Clinic; 2021. Available from: https://consultqd.clevelandclinic.org/the-truth-about-utis-in-older-adults/.
[15] Detweiler K, Mayers D, Fletcher SG. Bacteruria and urinary tract infections in the elderly. Urol Clin North Am 2015;42:561–8. https://doi.org/10.1016/j.ucl.2015.07.002.
[16] Neugent ML, Hulyalkar NV, Nguyen VH, Zimmern PE. Urinary microbiome: implications for urinary tract infection pathogenesis and treatment. Urol Clin North Am 2020;47:205–20. https://doi.org/10.1016/j.ucl.2019.12.004.
[17] Shvartzman P, Nasri Y. Urine culture collected from gel-based diapers: developing a novel experimental laboratory method. J Am Board Fam Pract 2004;17:91–5. https://doi.org/10.3122/jabfm.17.2.91.
[18] Sung WH, Liu CY, Yang CY, Chen CH, Tsao YT, Shen CF, et al. Urinalysis using a diaper-based testing device. Biosensors 2020;10:94. https://doi.org/10.3390/bios10080094.
[19] Paalanne N, Wikstedt L, Pokka T, Salo J, Uhari M, Renko M, et al. Diaper-embedded urine test device for the screening of urinary tract infections in children: a cohort study. BMC Pediatr 2020;20:1–7. https://doi.org/10.1186/s12887-020-02277-5.
[20] Lin SH, Kajiyama K, Wu T. Smart diaper: how it works. In: Proceedings of the 2017 ACM International Joint Conference on Pervasive and Ubiquitous Computing and Proceedings of the 2017 ACM International Symposium on Wearable Computers. ACM; 2017. p. 129–32. https://doi.org/10.1145/3123024.3123167.
[21] Inami IK, Inoue N. Real-time PCR assay using specimens on filter disks as a template for detection of cytomegalovirus in urine. J Clin Microbiol 2007;45:1305–7. https://doi.org/10.1128/JCM.02502-06.
[22] Carpenter RE. Navigating the new norm: the FDA's final rule on laboratory developed tests (LDTs) and their impact on laboratory operations. Clin Microbiol Newsl 2025;46:e001. https://doi.org/10.1016/j.clinmicnews.2024.09.001.
[23] Vine T, Carpenter RE, Bridges D. Dataset for a validated method of non-invasive urine collection using sodium polyacrylate-based diapers for PCR detection of uropathogens. Data Brief 2024;57:111036. https://doi.org/10.1016/j.dib.2024.111036.
[24] Kessler HH, Mühlbauer G, Stelzl E, Daghofer E, Santner BI, Marth E. Fully automated nucleic acid extraction: MagNA Pure LC. Clin Chem 2001;47:1124–6. https://doi.org/10.1093/clinchem/47.6.1124.
[25] Schrader C, Schielke A, Ellerbroek L, Johne R. PCR inhibitors – occurrence, properties and removal. J Appl Microbiol 2012;113:1014–26. https://doi.org/10.1111/j.1365-2672.2012.05384.x.
[26] Huggett JF, Novak T, Garson JA, Green C, Morris-Jones SD, Miller RF, et al. Differential susceptibility of PCR reactions to inhibitors: an important and unrecognised phenomenon. BMC Res Notes 2008;1:70. https://doi.org/10.1186/1756-0500-1-70.
[27] In: Davis CP. Normal flora. editor. In: Baron S, editor. Medical microbiology. 4th ed. Galveston: University of Texas Medical Branch at Galveston; 1996. Available from: https://www.ncbi.nlm.nih.gov/books/NBK7617/.
[28] Almas S, Carpenter RE, Singh A, Rowan C, Tamrakar VK, Sharma R. Deciphering microbiota of acute upper respiratory infections: a comparative analysis of PCR and mNGS methods for lower respiratory trafficking potential. Adv Respir Med 2023;91:49–65. https://doi.org/10.3390/arm91010006.
[29] Hoorfar J, Malorny B, Abdulmawjood A, Cook N, Wagner M, Fach P. Practical considerations in the design of internal amplification controls for diagnostic PCR assays. J Clin Microbiol 2004;42:1863–8. https://doi.org/10.1128/jcm.42.5.1863-1868.2004.
[30] Graff LA. Graff's textbook of urinalysis and body fluids. Philadelphia: Wolters Kluwer; 2020.
[31] Hilt EE, McKinley K, Pearce MM, Rosenfeld AB, Zilliox MJ, Mueller ER, et al. Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. J Clin Microbiol 2014;52:871–6. https://doi.org/10.1128/jcm.02876-13.
[32] Geerlings SE. Clinical presentations and epidemiology of urinary tract infections. Microbiol Spectr 2016;4:1–11. https://doi.org/10.1128/microbiolspec.uti-0002-2012. UTI-0002-2012.
[33] Yang S, Rothman RE. PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. Lancet Infect Dis 2004;4:337–48. https://doi.org/10.1016/S1473-3099(04)01044-8.
[34] Mitchell SL, Loeb MB, D'Agata E. Challenges in assessing nursing home residents with advanced dementia for suspected urinary tract infections. J Am Geriatr Soc 2013;61:1203–8. https://doi.org/10.1111/jgs.12070.
[35] Festa RA, Luke N, Mathur M, Parnell L, Wang D. A test combining multiplex-PCR with pooled antibiotic susceptibility testing has high correlation with expanded urine culture for detection of live bacteria in urinary tract infections. Diagn Microbiol Infect Dis 2023;105:115780. https://doi.org/10.1016/j.diagmicrobio.2023.116015.
[36] Rak M, Barlič-Maganja D, Kavčič M. Comparison of molecular and culture method in diagnosis of prosthetic joint infection. FEMS Microbiol Lett 2013;343:42–9. https://doi.org/10.1111/1574-6968.12125.
[37] Beckett CL, Harbarth S, Huttner B. Special considerations of antibiotic prescription in the geriatric population. Clin Microbiol Infect 2015;21:120–6. https://doi.org/10.1016/j.cmi.2014.08.018.
[38] Dyar OJ, Beović B, Vlahović-Palčevski V, Verheij T, Pulcini C. How can we improve antibiotic prescribing in primary care? Expert Rev Anti Infect Ther 2016;14:403–13. https://doi.org/10.1586/14787210.2016.1151353.
[39] Devillé WLJM, Yzermans JC, Van Duijn NP. The urine dipstick test useful to rule out infections: a meta-analysis of the accuracy. BMC Urol 2004;4:4. https://doi.org/10.1186/1471-2490-4-4.
[40] Van der Zee A, Roorda L, Bosman G, Ossewaarde JM. Molecular diagnosis of urinary tract infections by semi-quantitative detection of uropathogens in a routine clinical hospital setting. PLoS One 2016;11:e0150755. https://doi.org/10.1371/journal.pone.0150755.
[41] Shaikh N, Hoberman A, Mattoo TK. Urinary tract infections in infants and children older than one month: clinical features and diagnosis. In:. UpToDate. Waltham, MA: UpToDate, Inc.; 2021. Available from: https://medilib.ir/uptodate/show/5990.
[42] In: Strausbaugh LJ. Epidemiology and prevention of infections in residents of long-term care facilities. editor. In: Mayhall CG, editor. Hospital epidemiology and infection control. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2004.
[43] Mitchell SL, Shaffer ML, Loeb MB, Givens JL, Habtemariam D, Kiely DK. Infection management and multidrug-resistant organisms in nursing home residents with advanced dementia. JAMA Intern Med 2014;174:1660–7. https://doi.org/10.1001/jamainternmed.2014.3918.
[44] Kahvecioglu D, Ramiah K, McMaughan D, McCarthy M. Multidrug-resistant organism infections in US nursing homes: a national study of prevalence, onset, and transmission across care settings. Infect Control Hosp Epidemiol 2014;35:918–22. https://doi.org/10.1086/677835.