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Data Article

Dataset for a validated method of non-invasive urine collection using sodium polyacrylate-based diapers for PCR detection of uropathogens

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ABSTRACT

This dataset provides information from a study that validates a method for non-invasive urine collection utilizing sodium polyacrylate-based diapers, designed for polymerase chain reaction (PCR) detection of uropathogens. The dataset includes samples from 17 participants, comprising seven clinically contrived samples inoculated with known uropathogens and ten samples collected from volunteers wearing sodium polyacrylate-based diapers. The study involved optimizing urine extraction from the diaper matrices, ensuring minimal loss of diagnostic sensitivity for downstream quantitative PCR (qPCR) analysis. The qPCR targeted 22 uropathogens, six fungal species, and 18 antimicrobial resistance (AMR) genes, providing comprehensive molecular characterization of urinary pathogens. Comparative analyses were performed between diaper-derived and standard urine samples, with data demonstrating high concordance in detection outcomes, despite a modest average reduction in qPCR sensitivity (ΔCt of -1.65). Matrix effects were evaluated to determine the impact of the diaper material on qPCR amplification efficiency. The dataset is valuable for further research into non-invasive diagnostic techniques for urinary tract infections (UTIs), par-

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ticularly for populations unable to provide midstream urine samples, such as infants and elderly individuals. Potential reuse of this dataset includes evaluating non-invasive urine collection efficacy, exploring the interaction between sample matrices and molecular diagnostics, and assessing the scalability of diaper-based sampling methods in diverse clinical settings.

Specifications Table

Subject	Molecular Biology, Clinical Diagnostics
Specific subject area	Non-invasive urine collection using sodium polyacrylate-based diapers for PCR-based detection of uropathogens in challenging patient populations.
Type of data	Table, Graph, Figure
Data collection	Processed, Analyzed Urine samples were collected using sodium polyacrylate-based diapers. Seven samples were inoculated with known uropathogens, while ten were collected from volunteers. Urine was extracted, treated with calcium chloride, and analyzed via qPCR targeting 22 uropathogens, 6 fungal species, and 18 AMR genes. Comparative analysis between standard urine and diaper-derived samples showed minimal impact from the diaper matrix ($\Delta Ct = -1.65$). Matrix effects showed no false positives. Data confirm the feasibility of this non-invasive method for pathogen detection, especially for patients unable to provide midstream urine.
Data source location	Institution: Advanta Genetics, LLC Address: 10,935 CR 159, Tyler, Texas 75,703 Country: United States Latitude and longitude: 31.9686 ° N, 99.9018 ° W
Data accessibility	Repository name: Harvard Dataverse Data identification number: 10.7910/DVN/XFNVUX Direct URL to data: https://doi.org/10.7910/DVN/XFNVUX Instructions for accessing these data: Data are accessible through the Harvard Dataverse repository at the provided DOI link https://doi.org/10.7910/DVN/XFNVUX
Related research article	Vine, T., Carpenter, R.E., Bridges, D. (manuscript submitted). A Validated Method for Non-Invasive Urine Collection in Sodium Polyacrylate-Based Diapers for PCR Detection of Uropathogens. Practical Laboratory Medicine

1. Value of the Data

- This dataset [1] provides a validated non-invasive urine collection method using sodium polyacrylate-based diapers optimized for qPCR detection of uropathogens. Researchers focusing on alternative diagnostic approaches for UTIs, particularly in populations where traditional midstream collection is impractical (e.g., infants or elderly individuals), can find this dataset invaluable. It supports the development of accessible diagnostic solutions for vulnerable groups and helps in adapting molecular diagnostics for non-invasive sample collection.
- The dataset includes qPCR results from a panel of 22 uropathogens, 6 fungal species, and 18 AMR genes, making it highly valuable for molecular diagnostics researchers. The breadth of targets allows for comprehensive analysis of pathogen detection capabilities in non-invasive samples, facilitating a better understanding of the potential and limitations of such collection methods for a wide range of pathogens and antimicrobial resistance markers.
- This dataset provides detailed information on the effects of the sodium polyacrylate diaper matrix on nucleic acid recovery and PCR amplification efficiency. Researchers studying non-

invasive sampling techniques or matrix effects in clinical diagnostics can reuse these data to evaluate how the diaper matrix influences qPCR sensitivity, offering insights that may inform the design of improved non-invasive collection devices and protocols.
- The dataset offers detailed methods for urine recovery from the sodium polyacrylate-based diaper matrix, including chemical treatments to release the absorbed urine. Researchers can use these standardized procedures to replicate or adapt the sample extraction methodology in similar studies involving non-invasive collection from other absorbent materials, ensuring consistency and reliability in sample preparation for downstream molecular diagnostics.
- The dataset features comparative analyses between standard midstream urine samples and diaper-derived samples, highlighting the concordance of qPCR detection results. This comparison serves as a benchmark for researchers exploring alternative urine collection methods, allowing them to assess the diagnostic reliability, sensitivity, and specificity of non-invasive approaches in contrast to conventional techniques, thereby validating new diagnostic methods.

2. Background

The motivation behind compiling this dataset was to address the challenges associated with traditional urine collection methods for diagnosing UTIs, particularly in populations where midstream clean-catch collection is impractical. Vulnerable groups, such as infants, the elderly, or individuals with neurodegenerative conditions, often face difficulties in providing urine samples through standard methods, necessitating an alternative approach that is both non-invasive and reliable. The theoretical background centers around developing a validated, non-invasive sampling method using sodium polyacrylate-based diapers. This approach facilitates urine collection while preserving the integrity of molecular diagnostic capabilities. The methodological focus was to optimize urine extraction from the diaper matrix and evaluate its suitability for qPCR analysis targeting uropathogens, fungal species, and antimicrobial resistance genes.

This complements the field by providing a detailed dataset, enabling researchers to assess the non-invasive collection method's reproducibility and utility in molecular diagnostics for UTIs, with potential applications in improving diagnostic accessibility for underserved populations.

3. Data Description

The dataset is organized into multiple sheets containing data relevant to the non-invasive urine collection study using sodium polyacrylate-based diapers for qPCR detection of uropathogens. The dataset is structured as follows:

Key: This sheet provides details on the master mix solutions used for qPCR analysis, listing the targeted uropathogens and the corresponding fluorescent labels (FAM, SUN, CY5) for each organism.

Sheet15: This sheet appears to contain raw experimental data organized by sample identifiers (e.g., RM1, RM2). It lists multiple entries in a tabular form, presumably representing different replicates or conditions.

Diaper Key: This sheet provides information on the diaper brands used for urine collection along with the specific uropathogens tested in each sample. It helps associate each sample with its respective experimental conditions, such as diaper type.

Sheet14: This sheet includes a summary of sample types, specifying the number of replicates for different experimental conditions, such as "Urine," "Urine in Diaper," "Worn (Un-Spiked)," and "Worn (Spiked)."

Spiked: The "Spiked" sheet includes data comparing the qPCR results of diaper-derived urine and conventional urine samples for each target organism. Columns include sample identifiers, Ct values for diaper-derived and urine samples, the ΔCt (difference in Ct values), and percentage differences (% Diff).

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)

Spike Targets: This sheet lists the organisms targeted in the study, along with Gram classification, shape, arrangement, and relevant biological categories. It provides background information on each microorganism for identification and context in the study.

Matrix Effects: The "Matrix Effects" sheet contains data for evaluating the impact of the diaper material on qPCR sensitivity. It includes Ct values for samples with different master mixes and% difference values, indicating how the matrix influenced qPCR results.

Matrix Effects Charts: This sheet includes the targets analyzed, corresponding diapers, and resulting Ct values from both diaper-derived samples and control samples (TE). It also includes acceptable range values, which are helpful for assessing experimental variability and matrix effects on sample analysis.

4. Experimental Design, Materials and Methods

4.1. Materials and methods

4.1.1. 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.

4.1.2. Samples

Urine samples (1 µL) from clinical specimens received from Advanta Genetics (Tyler, Texas; www.aalabs.com) were inoculated onto Blood Agar Plates (Remel^{TM}, TSA with Sheep Blood) and

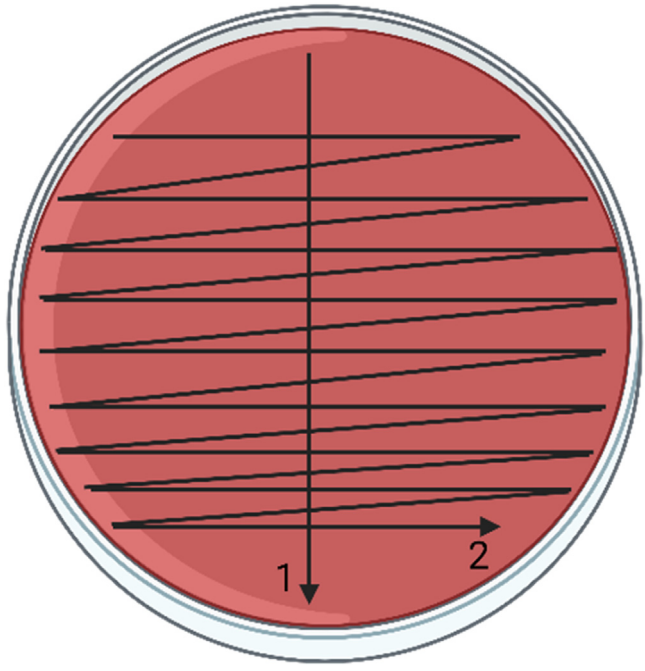

Fig. 1. Streaking pattern used for the isolation of uropathogens on Blood Agar Plates (Remel™ TSA with Sheep Blood) and CDC Anaerobic Blood Agar Plates (Remel™).

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 h with 5 % CO_2, while CDC plates were incubated anaerobically at 37 °C for 24–48 h 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 s 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.

4.1.3. Diaper spiking procedure

Urine samples (50 mL each) were collected from 7 healthy adult volunteers using a mid-stream clean-catch method in sterile specimen containers (LabAid™, Sterile Specimen Container with Temperature Strip). Each sample was inoculated with single colonies of pre-characterized uropathogens (Table 3), scraped from purity plates using a 1 µL inoculation loop. After inocu-

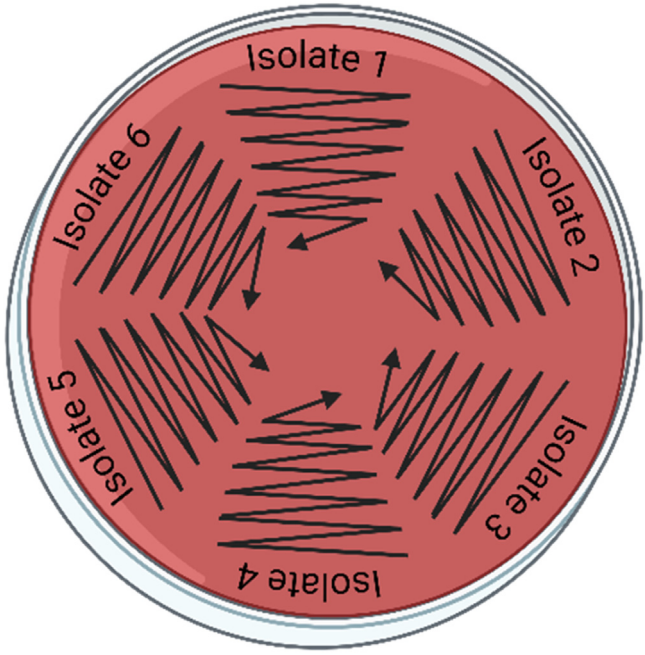

Fig. 2. Layout of purity plates used for verifying the isolation of colonies in the UTI diagnostic evaluation. Each segment illustrates the correct placement of colonies for subsequent purity verification and their relevance to downstream molecular testing.

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
E. 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

lation, the urine samples were sealed and vortexed for a minimum of 30 s at maximum speed using a Vortex-Genie 2 to ensure homogeneity.

For each urine sample, 25 mL was applied to the inner surface of various diaper brands (Table 3), simulating the soiling process. The remaining 25 mL were retained for qPCR analysis. The soiled diapers were left at room temperature for 4 h, followed by refrigeration for 24 h, mimicking clinical storage conditions where delays may occur before sample analysis. The remaining urine samples were similarly stored—4 h at room temperature followed by 24 h at

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	*E. coli*	FitRight® Underwear
6	*Proteus mirabilis*	Huggies® OverNites Diapers
7	*Streptococcus agalactiae, Prevotella bivia*	Pampers Baby-Dry

refrigerated temperatures—to maintain consistency in handling and conditions between paired diaper and urine samples.

4.1.4. 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 h 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. Following the wear period, the soiled diapers were placed in biohazard bags (Uline, 12×15″ Specimen Bags).

Upon receipt, the urine collected from a single elimination for each volunteer was entirely poured onto the internal surface of their corresponding diaper. The soiled diapers were then held at room temperature for 4 h, followed by refrigeration for 24 h to ensure consistency with the handling conditions used in the spiked diaper studies.

4.1.5. 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 g of calcium chloride (Thermo Fisher, Calcium Chloride, Anhydrous 93 %) was added to the container. The container was sealed and shaken vigorously to disperse the calcium chloride evenly, causing a slight increase in temperature detectable by hand.

After the calcium chloride 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 [2,3]. For a visual summary of this workflow, refer to Fig. 3.

4.1.6. 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 min to ensure thorough disruption of cells and efficient release of nucleic acids. Following lysis, 200

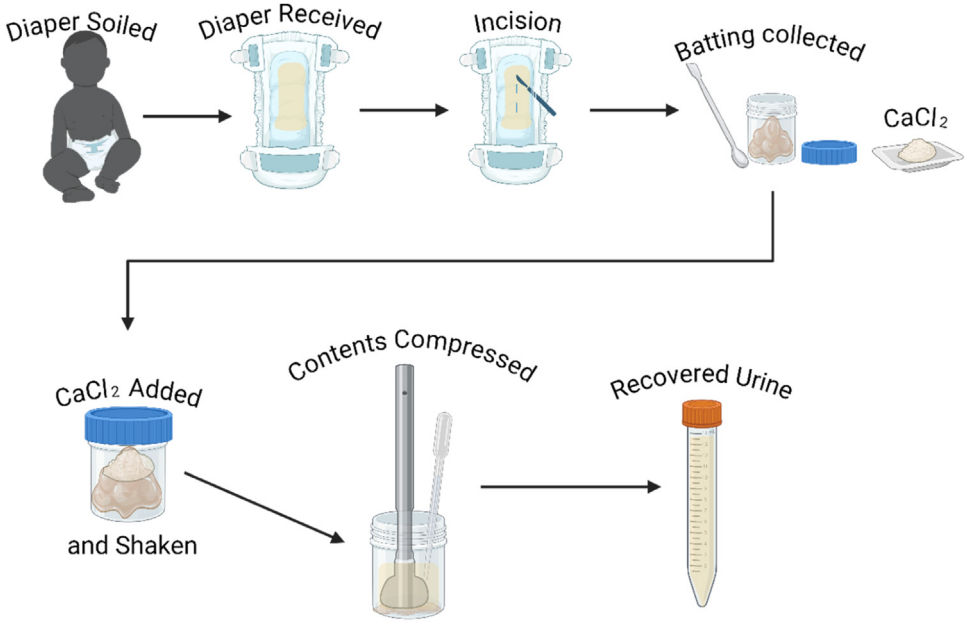

Fig. 3. Workflow depicting the steps involved in extracting urine from sodium polyacrylate-based diapers for PCR molecular diagnostics. Key steps include incision, batting transfer, calcium chloride treatment, and urine recovery to maintain nucleic acid integrity.

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.

4.1.7. 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 lower limit of detection (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 [4], which could otherwise reduce the assay's sensitivity at the LLoD [5]. 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 [6].

4.1.8. 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 di-

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	*E. 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 %

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1	RM1
B	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2	RM2
C	RM3	RM3	RM3	RM3	RM3	RM3	RM3	RM3	RM3	RM3	RM3	RM3	RM3	RM3	RM3	RM3	RM3	RM3	RM3	RM3	RM3	RM3	RM3	RM3
D	RM4	RM4	RM4	RM4	RM4	RM4	RM4	RM4	RM4	RM4	RM4	RM4	RM4	RM4	RM4	RM4	RM4	RM4	RM4	RM4	RM4	RM4	RM4	RM4
E	RM5	RM5	RM5	RM5	RM5	RM5	RM5	RM5	RM5	RM5	RM5	RM5	RM5	RM5	RM5	RM5	RM5	RM5	RM5	RM5	RM5	RM5	RM5	RM5
F	RM6	RM6	RM6	RM6	RM6	RM6	RM6	RM6	RM6	RM6	RM6	RM6	RM6	RM6	RM6	RM6	RM6	RM6	RM6	RM6	RM6	RM6	RM6	RM6
G	RM7	RM7	RM7	RM7	RM7	RM7	RM7	RM7	RM7	RM7	RM7	RM7	RM7	RM7	RM7	RM7	RM7	RM7	RM7	RM7	RM7	RM7	RM7	RM7
H	RM8	RM8	RM8	RM8	RM8	RM8	RM8	RM8	RM8	RM8	RM8	RM8	RM8	RM8	RM8	RM8	RM8	RM8	RM8	RM8	RM8	RM8	RM8	RM8
I	RM9	RM9	RM9	RM9	RM9	RM9	RM9	RM9	RM9	RM9	RM9	RM9	RM9	RM9	RM9	RM9	RM9	RM9	RM9	RM9	RM9	RM9	RM9	RM9
J	RM10	RM10	RM10	RM10	RM10	RM10	RM10	RM10	RM10	RM10	RM10	RM10	RM10	RM10	RM10	RM10	RM10	RM10	RM10	RM10	RM10	RM10	RM10	RM10
K	RM11	RM11	RM11	RM11	RM11	RM11	RM11	RM11	RM11	RM11	RM11	RM11	RM11	RM11	RM11	RM11	RM11	RM11	RM11	RM11	RM11	RM11	RM11	RM11
L	RM12	RM12	RM12	RM12	RM12	RM12	RM12	RM12	RM12	RM12	RM12	RM12	RM12	RM12	RM12	RM12	RM12	RM12	RM12	RM12	RM12	RM12	RM12	RM12
M	RM13	RM13	RM13	RM13	RM13	RM13	RM13	RM13	RM13	RM13	RM13	RM13	RM13	RM13	RM13	RM13	RM13	RM13	RM13	RM13	RM13	RM13	RM13	RM13
N	RM14	RM14	RM14	RM14	RM14	RM14	RM14	RM14	RM14	RM14	RM14	RM14	RM14	RM14	RM14	RM14	RM14	RM14	RM14	RM14	RM14	RM14	RM14	RM14
O	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15	RM15
P	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 the PCR reaction mixtures as applied to the 384-well plate for qPCR analysis of uropathogens and antimicrobial resistance genes. The diagram shows the arrangement of positive and negative controls to ensure assay reliability.

aper 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 [5]. 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 predesigned 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 (Table 4).

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. 5) [6,7] (Fig. 6).

Limitations

While this study demonstrated the feasibility of using sodium polyacrylate-based diapers for molecular UTI diagnostics, several limitations should be noted. First, diaper materials may introduce inhibitors or reduce sample integrity, leading to increased Ct values and slightly reduced

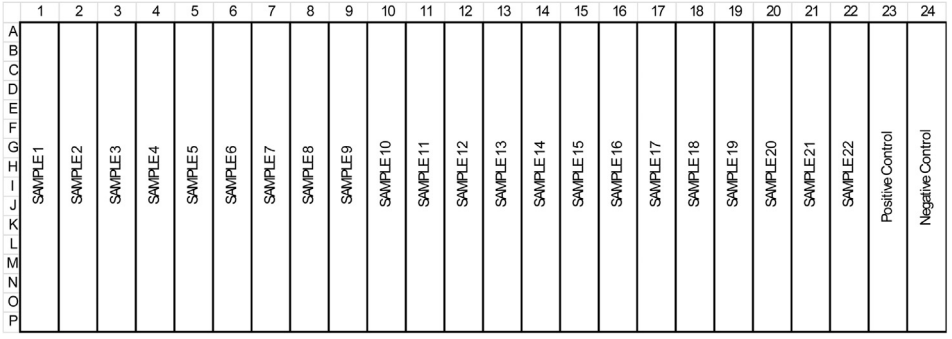

Fig. 5. Schematic of the sample layout in a 384-well plate used for qPCR, including the positions of positive amplification control and negative amplification control, crucial for maintaining the consistency and validity of qPCR results.

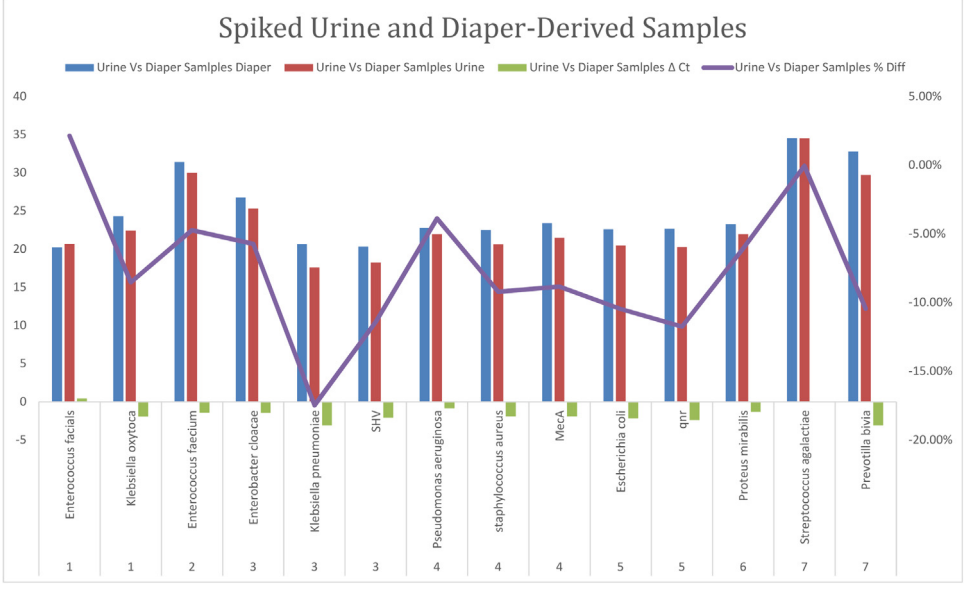

Fig. 6. Graphical comparison of qPCR detection efficiency for uropathogens in spiked urine samples versus diaper-derived samples. The figure highlights Ct value differences between sample types, demonstrating concordance and matrix effect influences.

sensitivity compared to clean-catch urine, particularly at low pathogen concentrations [8]. 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 [10]. 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 [11]. 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 [12].

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).

CRediT Author Statement

Tyler Vine: Experiment planning and execution, Sample preparation and experimentation, Data curation, writing. **Rob E. Carpenter:** Conceptualization, writing-reviewing and editing. **Debbie Bridges:** writing-reviewing and editing.

Data Availability

Sodium Polyacrylate-Based Diapers for PCR Detection of Uropathogens (Original data) (Harvard Dataverse).

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Declaration of Competing Interest

There is no conflict of interests.

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Further Reading

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