White Paper: Direct-to-PCR (D2P) Extraction-Free Technology

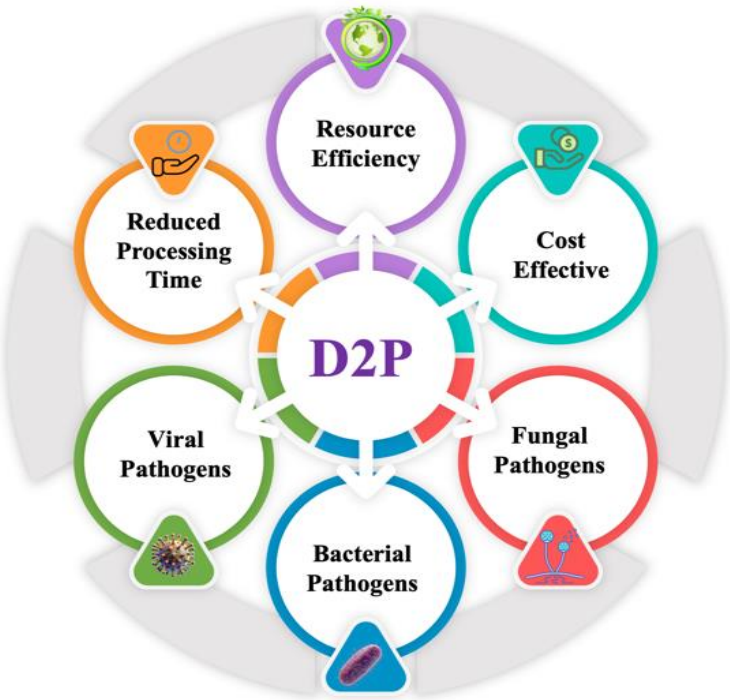
Study Highlights

Broad Pathogen Coverage

This study assesses the Direct-to-PCR (D2P) extraction-free PCR method for detecting pathogens linked to urinary tract infections (UTIs), sexually transmitted infections (STIs), and respiratory tract infections (RTIs). The findings highlight its robust performance in identifying a wide range of pathogens, including bacteria, fungi, and viruses.

Comparable Diagnostic Performance

The D2P extraction-free method achieves sensitivity and specificity equivalent to traditional silica column- and magnetic bead-based extraction techniques. It exhibits minimal differences in cycle threshold (Ct) values (typically $\Delta Ct \leq 1.5$), ensuring reliable and accurate pathogen detection.



***Significant Time Reduction*:** The D2P extraction-free method reduces sample processing time from ~120 minutes to 45 minutes, enabling faster diagnostics and improved workflow efficiency in clinical laboratories.

Cost-Effective and Scalable: The D2P extraction-free method lowers per-sample costs by eliminating the need for proprietary reagents and specialized equipment. It is well-suited for high-throughput laboratories and resource-limited settings.

Reduced Contamination Risk: The streamlined, D2P extraction-free workflow reduces manual handling, significantly lowering the risk of cross-contamination and nucleic acid degradation. This ensures greater reliability and consistency in diagnostic results.

Robust Performance Across Challenging Pathogens: The D2P extraction-free method delivers exceptional performance, effectively lysing both easily extracted pathogens like *Escherichia coli* and more challenging ones such as *Staphylococcus aureus*, *Candida auris*, single-stranded RNA viruses, and double-stranded DNA viruses like Herpes Simplex Virus (HSV). Its ability to handle a diverse range of pathogens—both those with simpler and more resilient cell structures—demonstrates its versatility and reliability.

Infectious Disease Detection with Simplified Molecular Diagnostics

Molecular diagnostic tools, including polymerase chain reaction (PCR) and quantitative PCR (qPCR), have revolutionized the detection of infectious diseases by providing rapid, precise pathogen identification through genetic analysis. However, traditional diagnostic workflows are hindered by significant challenges such as long turnaround times, labor-intensive pre-processing, high nucleic acid extraction costs, and the need for specialized expertise. These barriers are particularly problematic in resource-limited settings, where quick and accurate diagnostics are crucial. The limitations of conventional microbiology methods compared to modern qPCR approaches are clear. Culture-based methods take 24–48 hours to yield results, while traditional qPCR workflows involve lengthy multi-step extraction processes, often taking around four hours.

The D2P extraction-free approach breaks through these limitations. By eliminating the need for extraction and drastically reducing pre-processing steps to under 20 minutes, D2P extraction-free delivers results in just 90 minutes. This innovation not only maintains exceptional sensitivity (>95%) but also significantly reduces costs. It represents more than just an incremental improvement—it is a game-changing advancement that enhances diagnostic efficiency, scalability, and accessibility. This is particularly valuable in time-sensitive and cost-conscious clinical and research settings. With the D2P extraction-free PCR, the future of infectious disease detection is faster, more affordable, and more accessible than ever before.

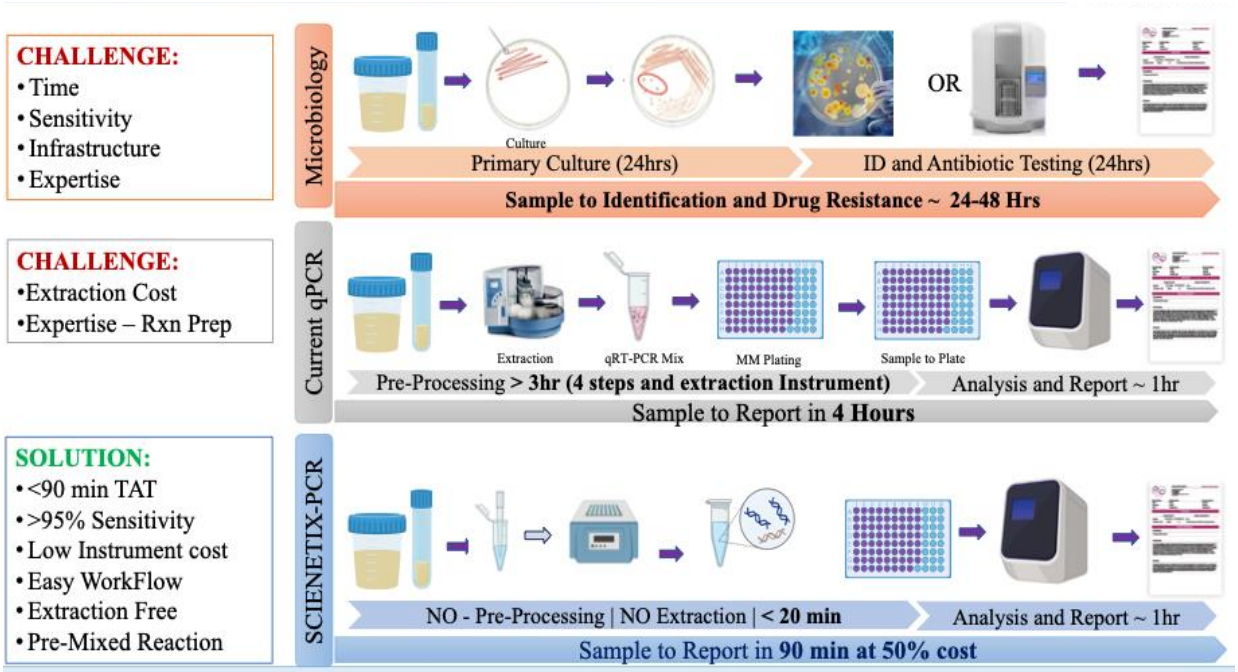


Figure 1. *Accelerated testing time compared to standard diagnostic methods*

D2P Extraction-Free vs Conventional Extraction: A Performance Comparison

Figure 2 compares the nucleic extraction performance of microbial isolates across various microorganisms, including Gram-negative bacteria, fungi, RNA viruses, and DNA viruses. The results show that the D2P extraction-free method demonstrates performance that is comparable to or slightly better than both QIAGEN and KingFisher in most categories.

Key observations include:

1. **DNA Viruses (HSV and HAdV):** the D2P extraction-free method performs strongly, with extraction values around 30 units for HSV and 27-28 units for HAdV.

2. **Gram-negative Bacteria (E. coli, K. pneumoniae, and N. gonorrhoeae):** the D2P extraction-free method shows consistent performance in the range of 20-25 units.

3. **RNA Viruses (COV 229E and ParaFlu):** the D2P extraction-free method achieves slightly higher extraction efficiency compared to traditional methods.

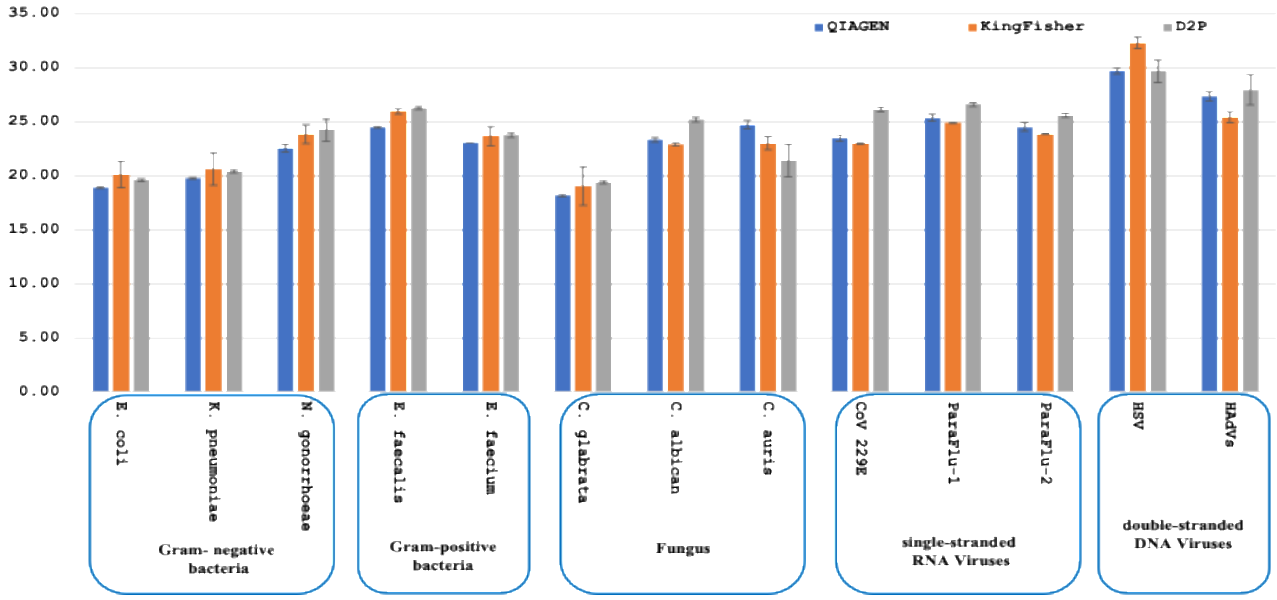


Figure 2. *A comparative analysis of QIAGEN, KingFisher, and D2P nucleic acid extraction methods*

A comparative analysis (Table 1) was conducted to evaluate the performance of QIAGEN, KingFisher, and the D2P extraction-free method. This analysis utilized a panel of well-characterized microbial reference isolates, representing a diverse range of bacteria, fungi, and viruses.

Table 1. *A comparison of Ct values for various microorganisms across different extraction methods*

Microorganisms		Mean±Ct value across Different extraction methods			Ct value difference		
Type	Pathogens	QIAGEN	KingFisher	D2P	$\Delta Ct^{(KF-QI)}$	$\Delta Ct^{(D2P-QI)}$	$\Delta Ct^{(KF-D2P)}$
Gram (-) Bacteria	*E. coli*	18.93±0.08	20.11±1.2	19.6±0.11	1.18	0.67	0.51
	K. pneumoniae	19.76±0.09	20.65±1.51	20.42±0.12	0.88	0.65	0.23
	N. gonorrheae	22.54±0.34	23.84±0.88	24.24±1.06	1.29	1.69	-0.4
Gram (+) Bacteria	*E. faecalis*	24.47±0.09	25.98±0.26	26.27±0.13	1.50	1.79	-0.29
	E. faecium	23.08±0.00	23.67±0.91	23.77±0.18	0.58	0.68	-0.1
Fungus	*C. glabrata*	18.19±0.11	19.06±1.76	19.40±0.12	0.86	1.20	-0.34
	C. albicans	23.34±0.25	22.92±0.14	25.2±0.22	-0.42	1.85	-2.28
	C. auris	24.73±0.41	23±0.61	21.39±1.5	-1.73	-3.34	1.61
ssRNA Virus	CoV 229E	23.48±0.29	22.98±0.1	26.11±0.2	-0.49	2.64	-3.13
	ParaFlu-1	25.37±0.31	24.88±0.06	26.61±0.17	-0.48	1.24	-1.73
	ParaFlu-2	24.52±0.42	23.87±0.06	25.58±0.18	-0.65	1.07	-1.71
dsDNA virus	HSV	29.73±0.30	32.3±0.53	29.68±1.04	2.58	-0.05	2.62
	Adenovirus	27.34±0.43	25.43±0.5	27.95±1.36	-1.91	0.61	-2.52

Note: QI = QIAGEN; KF = KingFisher; D2P = Direct-to-PCR; ssRNA = Single-stranded RNA; dsRNA = Double-stranded DNA; Cov 229E = Coronavirus 229E; HSV = Herpes Simplex Virus; Ct = Cycle Threshold

The D2P extraction-free method achieved equivalent diagnostic accuracy across diverse microbial isolates as demonstrated by the following findings:

Bacterial Pathogens:

- Gram-negative bacteria: ΔCt values ranged from 0.23 to 1.69 compared to the gold standard.
- Gram-positive bacteria: ΔCt values ranged from 0.58 to 1.79 compared to the gold standard.

Fungal Pathogens:

- *Candida auris*: Exceptional performance with a ΔCt of -3.34 compared to the gold standard.
- *Candida glabrata*: Comparable performance with a ΔCt of 1.20.

Viral Pathogens:

- RNA viruses: ΔCt values ranged from 1.07 to 2.64 compared to the gold standard.
- DNA viruses: Comparable performance for DNA viruses with a ΔCt of -0.05.

D2P Extraction-Free Performance in UTI, STI, and RPI Clinical Samples

Table 2 summarizes the diagnostic accuracy of the D2P extraction-fee method in clinical samples from various infection types, including UTIs, STIs, and RTIs. The performance of the D2P extraction-free method was evaluated based on key metrics: sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV).

Table 2. *Diagnostic accuracy of the D2P extraction-free method in clinical samples*

Infection Type	Sample Size (n)	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV	NPV
UTI	40	96.21	99.33	99.33	0.98	0.99
STI	24	98.22	97.83	97.83	0.95	0.99
RPI	52	97.51	98.95	98.95	0.96	0.99

The results demonstrate that the D2P extraction-free method offers high diagnostic accuracy, with sensitivity and specificity exceeding 95% across all infection types. The D2P extraction-free method demonstrated excellent diagnostic accuracy in clinical samples, effectively detecting a wide range of pathogens across various infection types. These findings underscore the D2P extraction-free method's reliability and robust performance in real-world clinical settings, ensuring accurate pathogen identification across diverse sample types. The method's high efficiency and consistent results further position the D2P extraction-free method as a strong alternative to traditional extraction techniques, offering both clinical value and operational efficiency.

UTI Sample Analysis with the D2P extraction-free method

De-identified residual samples (n=40) from patients with suspected UTIs were processed using both the KingFisher bead-based extraction method and the D2P extraction-free approach (Table 3). After extraction, nucleic acids from both methods were subjected to a preformulated, organism-specific qPCR assay designed to target and quantify the respective pathogens. The results obtained from the D2P extraction-free method were compared to those from the KingFisher method, with each D2P extraction-free method sample classified as true positive, true negative, false positive, or false negative, based on the KingFisher baseline results.

Table 3. *A comparative analysis of UTI pathogen detection: Bead-based (KingFisher) vs. D2P extraction-free method*

Microorganisms	TP	TN	FP	FN	Sensitivity	Specificity	Accuracy	PPV	NPV
P. mirabilis	7	33	0	0	100.00%	100.00%	100.00%	1.00	1.00
E. coli	26	14	0	0	100.00%	100.00%	100.00%	1.00	1.00
P. bivia	8	32	0	0	100.00%	100.00%	100.00%	1.00	1.00
K. pneumoniae	14	25	0	1	93.33%	100.00%	100.00%	1.00	0.96
S. agalactiae (GBS)	6	34	0	0	100.00%	100.00%	100.00%	1.00	1.00
S. pyogenes (GAS)	0	40	0	0	NA	100.00%	100.00%	0.00	1.00
A. baumannii	2	38	0	0	100.00%	100.00%	100.00%	1.00	1.00
E. cloacae	13	26	0	1	92.86%	100.00%	100.00%	1.00	0.96
E. faecalis	20	18	0	2	90.91%	100.00%	100.00%	1.00	0.90
E. faecium	5	35	0	0	100.00%	100.00%	100.00%	1.00	1.00
C. parapsilosis	0	40	0	0	NA	100.00%	100.00%	0.00	1.00
C. tropicalis	6	34	0	0	100.00%	100.00%	100.00%	1.00	1.00
P. aeruginosa	14	26	0	0	100.00%	100.00%	100.00%	1.00	1.00
C. glabrata	9	30	1	0	100.00%	96.77%	96.77%	0.90	1.00
RNAseP	34	4	0	2	94.44%	100.00%	100.00%	1.00	0.67
S. aureus	4	35	1	0	100.00%	97.22%	97.22%	0.80	1.00
C. albicans	8	31	0	1	88.89%	100.00%	100.00%	1.00	0.97
T. rubrum	0	40	0	0	NA	100.00%	100.00%	0.00	1.00
E. floccosum	0	40	0	0	NA	100.00%	100.00%	0.00	1.00
C. auris	0	40	0	0	NA	100.00%	100.00%	0.00	1.00
Citrobactor	2	38	0	0	100.00%	100.00%	100.00%	1.00	1.00
B. fragilis	6	34	0	0	100.00%	100.00%	100.00%	1.00	1.00
A. schaalii	8	31	0	1	88.89%	100.00%	100.00%	1.00	0.97
P. vulgaris	2	38	0	0	100.00%	100.00%	100.00%	1.00	1.00
S. marcescens	0	40	0	0	NA	100.00%	100.00%	0.00	1.00
K. oxytoca	2	37	1	0	100.00%	97.37%	97.37%	0.67	1.00
S. saprophyticus	1	39	0	0	100.00%	100.00%	100.00%	1.00	1.00

Microorganisms	TP	TN	FP	FN	Sensitivity	Specificity	Accuracy	PPV	NPV
M. morganii	5	35	0	0	100.00%	100.00%	100.00%	1.00	1.00
S. haemolyticus	11	26	2	1	91.67%	92.86%	92.86%	0.85	0.96
EEC	38	2	0	0	100.00%	100.00%	100.00%	1.00	1.00
A. urinae	2	38	0	0	100.00%	100.00%	100.00%	1.00	1.00
K. aerogenes	3	37	0	0	100.00%	100.00%	100.00%	1.00	1.00
VanB	0	40	0	0	NA	100.00%	100.00%	0.00	1.00
DfrA1	5	35	0	0	100.00%	100.00%	100.00%	1.00	1.00
MecA	10	29	0	1	90.91%	100.00%	100.00%	1.00	0.97
VanM	0	40	0	0	NA	100.00%	100.00%	0.00	1.00
qnrAS	6	32	1	1	85.71%	96.97%	96.97%	0.85	0.96
gyrA	0	40	0	0	NA	100.00%	100.00%	0.00	1.00
CTX-M-Grp1	10	29	1	0	100.00%	96.67%	96.67%	0.91	1.00
SHV	12	27	0	1	92.31%	100.00%	100.00%	1.00	0.96
TEM	14	25	0	1	93.33%	100.00%	100.00%	1.00	0.96
NDM	0	40	0	0	NA	100.00%	100.00%	0.00	1.00
RNAseP	32	7	1	0	100.00%	87.50%	87.50%	0.97	1.00
VanA	9	29	1	1	90.00%	96.67%	96.67%	0.90	0.96
TetB	6	34	0	0	100.00%	100.00%	100.00%	1.00	1.00
Sul1	17	21	1	1	94.44%	95.45%	95.45%	0.94	0.95
TetM	29	10	0	1	96.67%	100.00%	100.00%	1.00	0.91
OXA-48	0	40	0	0	NA	100.00%	100.00%	0.00	1.00
Total	406	1488	10	16	96.21%	99.33%	99.33%	0.98	0.99

Note: TP = True Positive; TN = True Negative; FP = False Positive; FN = False Negative; PPV = Positive Predictive Value; NPV = Negative Predictive Value; RNAseP = Ribonuclease P; EEC = Exogenous extraction control; VanB, VanM, VanA = Vancomycin Resistance Genes; DfrA1 = Dihydrofolate Reductase Gene A1; MecA = Methicillin Resistance Gene A; qnrAS = Quinolone Resistance Genes A/S; gyrA = DNA Gyrase Subunit A Mutation; CTX-M-Grp1 = Extended-Spectrum Beta-Lactamase Gene Group 1; SHV = Sulfhydryl Variable Beta-Lactamase; TEM = Temoniera Beta-Lactamase; NDM = New Delhi Metallo-Beta-Lactamase; TetB, TetM = Tetracycline Resistance Genes B/M; Sul1 = Sulfonamide Resistance Gene 1; OXA-48 = Oxacillinase-48 Beta-Lactamase; NA = performance metrics are not applicable due to the absence of data.

Results from clinical UTI samples demonstrate that the D2P extraction-free method provides excellent diagnostic performance, with sensitivity and specificity consistently above 90% for most UTI pathogens. The D2P extraction-free method showed comparable or superior performance to the bead-based KingFisher method, even for more challenging pathogens like *Candida species* and *Enterococcus species*. This comparative analysis underscores the potential of the D2P extraction-free method as a faster, more cost-effective alternative to traditional extraction-based techniques, offering enhanced scalability and ease of use in clinical settings. With its high diagnostic accuracy, the D2P extraction-free method is well-suited for routine clinical use and rapid pathogen identification in UTI diagnostics.

STI Sample Analysis with the D2P extraction-free method

De-identified residual samples (n=24) from patients with suspected STIs were processed using both the KingFisher bead-based extraction method and the D2P extraction-free approach (Table 4). After extraction, nucleic acids from both methods were analyzed using a preformulated, organism-specific qPCR assay tailored to detect and quantify the relevant pathogens. The results from the D2P extraction-free method were then compared with those from the KingFisher method, categorizing each D2P extraction-free method sample as true positive, true negative, false positive, or false negative, based on the KingFisher baseline results.

Table 4. *A comparative analysis of STI pathogen detection: Bead-based (KingFisher) vs. D2P extraction-free method*

Microorganisms	TP	TN	FP	FN	Sensitivity	Specificity	Accuracy	PPV	NPV
Neisseria gonorrhoeae	4	20	0	0	100.00%	100.00%	100.00%	1.00	1.00
Trichomonas vaginalis	1	23	0	0	100.00%	100.00%	100.00%	1.00	1.00
Chlamydia trachomatis	0	0	0	0	NA	NA	NA	NA	NA
Herpes Simplex Virus 1 and 2	6	18	0	0	100.00%	100.00%	100.00%	1.00	1.00
Treponema pallidum	5	18	1	0	100.00%	94.74%	94.74%	0.83	1.00
Exogenous Extraction Control	4	20	0	0	100.00%	100.00%	100.00%	1.00	1.00
GAPDH (Control)	0	0	0	0	NA	NA	NA	NA	NA
Haemophilus ducreyi	5	19	0	0	100.00%	100.00%	100.00%	1.00	1.00
Atopobium vaginae	13	10	1	0	100.00%	90.91%	90.91%	0.93.	1.00
Megasphaera type II	0	0	0	0	NA	NA	NA	NA	NA
BVAB- 2	10	13	0	1	90.91%	100.00%	100.00%	1.00	0.91
BVAB- 1	5	19	0	0	100.00%	100.00%	100.00%	1.00	1.00
Mycoplasma genitalium	5	19	0	0	100.00%	100.00%	100.00%	1.00	1.00
Gardnerella vaginalis	19	3	0	0	100.00%	100.00%	100.00%	1.00	1.00
Mobiluncus curtisii	1	22	1	0	100.00%	95.65%	95.65%	0.50	1.00
Ureaplasma urealyticum/parvum	6	16	2	0	100.00%	88.89%	88.89%	0.75	1.00
BVAB-3	4	20	0	0	100.00%	100.00%	100.00%	1.00	1.00
Mobiluncus mulieris	9	13	1	1	90.00%	92.86%	92.86%	0.90	0.93
Megasphaera type I	6	18	0	0	100.00%	100.00%	100.00%	1.00	1.00
Lactobacillus	8	15	0	1	88.89%	100.00%	100.00%	1.00	0.94
Total	111	286	6	3	98.22%	97.83%	97.83%	0.95	0.99

Note: TP = True Positive; TN = True Negative; FP = False Positive; FN = False Negative; PPV = Positive Predictive Value; NPV = Negative Predictive Value; GAPDH - glyceraldehyde-3-phosphate dehydrogenase; BVAB = Bacterial Vaginosis-Associated Bacteria; NA = performance metrics are not applicable due to the absence of data.

Results from clinical STI samples demonstrate that the D2P extraction-free method demonstrates high sensitivity, specificity, and accuracy for most STI pathogens. Both the KingFisher and the D2P extraction free methods achieved perfect sensitivity and specificity (100%) for *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Herpes Simplex Virus 1 and 2*, and *Haemophilus ducreyi*. Additionally, *Treponema pallidum* and *Gardnerella vaginalis* showed excellent performance with both methods. While *Mobiluncus curtisii* showed a slightly lower positive predictive value (PPV) of 0.50, the overall results highlight the D2P extraction-free method as a reliable, efficient method for STI pathogen detection with high diagnostic accuracy.

Respiratory Pathogen (RP) Detection with the D2P extraction-free method

De-identified residual samples (n=52) from patients with suspected RTIs were processed using both the KingFisher bead-based extraction method and the D2P extraction-free method (Table 5). After extraction, nucleic acids from each method were analyzed using a preformulated, organism-specific qPCR assay designed to target and quantify the relevant respiratory pathogens. The results obtained from the D2P extraction-free method were then compared to those from the KingFisher method, with each D2P extraction-free method sample categorized as true positive, true negative, false positive, or false negative, based on the KingFisher baseline results.

Table 5. *A comparative analysis of RTI pathogen detection: Bead-Based (KingFisher) vs. extraction-free method*

Microorganisms	TP	TN	FP	FN	Sensitivity	Specificity	Accuracy	PPV	NPV
HCoV-229E	4	48	0	0	100.00%	100.00%	100.00%	1.00	1.00
HCoV-HKU1	1	51	0	0	100.00%	100.00%	100.00%	1.00	1.00
HCoV-OC43	4	48	0	0	100.00%	100.00%	100.00%	1.00	1.00
HCoV-NL63	15	36	0	1	93.75%	100.00%	100.00%	1.00	0.97
HHV-4	0	0	0	0	NA	NA	NA	NA	NA
HPeV	0	0	0	0	NA	NA	NA	NA	NA
HAdV-B C	0	0	0	0	NA	NA	NA	NA	NA
HBoV	0	0	0	0	NA	NA	NA	NA	NA
HPIV-1	4	48	0	0	100.00%	100.00%	100.00%	1.00	1.00
HPIV-2	0	0	0	0	NA	NA	NA	NA	NA
HPIV-4	6	45	1	0	100.00%	97.83%	97.83%	0.86	1.00
HPIV-3	8	41	2	1	88.89%	95.35%	95.35%	0.80	0.98
RNAseP	52	0	0	0	100.00%	NA	NA	1.00	0.00
Flu A H1N1 swl	0	0	0	0	NA	NA	NA	NA	NA
HRV-C	1	51	0	0	100.00%	100.00%	100.00%	1.00	1.00
RSV A/B	2	50	0	0	100.00%	100.00%	100.00%	1.00	1.00
Flu A	1	50	1	0	100.00%	98.04%	98.04%	0.50	1.00
Flu B	10	41	1	0	100.00%	97.62%	97.62%	0.91	1.00
EEC	34	18	0	0	100.00%	100.00%	100.00%	1.00	1.00
SARS-CoV-2	10	41	1	0	100.00%	97.62%	97.62%	0.91	1.00

Microorganisms	TP	TN	FP	FN	Sensitivity	Specificity	Accuracy	PPV	NPV
EV-A71	1	51	0	0	100.00%	100.00%	100.00%	1.00	1.00
HMPV	1	51	0	0	100.00%	100.00%	100.00%	1.00	1.00
ERTC	43	9	0	0	100.00%	100.00%	100.00%	1.00	1.00
EV-D68	0	0	0	0	NA	NA	NA	NA	NA
B. parapertussis	4	48	0	0	100.00%	100.00%	100.00%	1.00	1.00
B. pertussis/holmesii	4	48	0	0	100.00%	100.00%	100.00%	1.00	1.00
K. pneumoniae	6	46	0	0	100.00%	100.00%	100.00%	1.00	1.00
C. pneumoniae	0	0	0	0	NA	NA	NA	NA	NA
M. catarrhalis	21	27	2	2	91.30%	93.10%	93.10%	0.91	0.93
MecA	32	18	0	2	94.12%	100.00%	100.00%	1.00	0.90
S. aureus	9	42	0	1	90.00%	100.00%	100.00%	1.00	0.98
Van A/B	5	47	0	0	100.00%	100.00%	100.00%	1.00	1.00
H. influenzae	15	33	2	1	93.75%	94.29%	94.29%	0.88	0.97
Hib	2	49	1	0	100.00%	98.00%	98.00%	0.50	1.00
M. pneumoniae	0	0	0	0	NA	NA	NA	NA	NA
S. typhi/paratyphi	4	47	0	0	100.00%	100.00%	100.00%	1.00	1.00
S. pyogenes (GAS)	5	46	1	0	100.00%	97.87%	97.87%	0.83	1.00
Legionella spp	0	0	0	0	NA	NA	NA	NA	NA
S. pneumoniae	8	44	0	0	100.00%	100.00%	100.00%	1.00	1.00
S. agalactiae	1	50	1	0	100.00%	98.04%	98.04%	0.50	1.00
Total	313	1224	13	8	97.51%	98.95%	98.95%	0.96	0.99

Note: TP = True Positive; TN = True Negative; FP = False Positive; FN = False Negative; PPV = Positive Predictive Value; NPV = Negative Predictive Value; HCoV-229E = Human Coronavirus 229E; HCoV-HKU1 = Human Coronavirus; HKU1 = HCoV-OC43 = Human Coronavirus OC43; HCoV-NL63 = Human Coronavirus NL63; HHV-4 = Human Herpesvirus 4 (Epstein-Barr Virus); = HPeV = Human Parechovirus; HAdV-B C = Human Adenovirus species B and C; HBoV = Human Bocavirus; HPIV-1 = Human Parainfluenza Virus type 1; HPIV-2 = Human Parainfluenza Virus type 2; = HPIV-3 = Human Parainfluenza Virus type 3 = HPIV-4 = Human Parainfluenza Virus type 4; = RNAseP = Ribonuclease P; Flu A H1N1 swl = Influenza A H1N1 (Swine Lineage); HRV-C = Human Rhinovirus C; RSV A/B = Respiratory Syncytial Virus subtypes A and B; = Flu A = Influenza A; Flu B = Influenza B; EEC = Exogenous Extraction Control; SARS-CoV-2 = Severe Acute Respiratory Syndrome Coronavirus 2; EV-A71 = Enterovirus A71 = HMPV = Human Metapneumovirus; ERTC = Endogenous Reference Target Control; EV-D68 = Enterovirus D68; MecA = gene associated with methicillin resistance in *Staphylococcus aureus*; Van A/B = Vancomycin resistance genes A and B; Hib = *Haemophilus influenzae* type B; NA = performance metrics are not applicable due to the absence of data.

The comparative analysis of RTI pathogen detection using the bead-based KingFisher method and the D2P extraction-free method shows excellent diagnostic performance across most pathogens. Both methods demonstrated 100% sensitivity and specificity for several respiratory pathogens, including HCoV-229E, HCoV-HKU1, HPIV-1, RSV A/B, and SARS-CoV-2. The overall accuracy of 98.95% and high PPV further confirm the reliability of the D2P extraction-free method in diagnosing respiratory tract infections.

The Future of Molecular Diagnostic Testing

In today's fast-paced healthcare environment, the need for rapid and accurate diagnostics is critical. The Direct-to-PCR (D2P) extraction-free method offers a significant advancement in molecular diagnostics by reducing processing time, simplifying workflows, and eliminating the need for costly extraction procedures. This innovative technique allows diagnostic laboratories to achieve greater efficiency and cost-effectiveness while maintaining high diagnostic accuracy.

The D2P extraction-free method demonstrates diagnostic performance comparable to gold-standard extraction techniques, with minimal differences in cycle threshold (Ct) values ($\Delta Ct \leq 1.5$) across a broad spectrum of pathogens, including bacteria, fungi, and viruses. By streamlining sample preparation and reducing manual handling, the method minimizes the risk of cross-contamination and nucleic acid degradation, enhancing reliability and reproducibility.

In resource-limited settings where rapid turnaround and accuracy are paramount, the D2P extraction-free method provides a scalable and robust solution. Its compatibility with high-throughput workflows and potential for automation makes it well-suited for point-of-care applications, improving the timeliness of clinical decision-making and patient outcomes.

Future Potential

The D2P extraction-free method is poised to revolutionize molecular diagnostics through its adaptability to automated platforms and diverse sample types, including challenging matrices such as blood and stool. Continued advancements in inhibitor-tolerant enzymes will further expand the utility of this approach, facilitating rapid, accessible, and reliable diagnostics. This method represents a transformative step toward more efficient, cost-effective, and scalable diagnostic solutions, meeting the evolving demands of modern healthcare.

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