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Posted Date: 3 April 2025

doi: 10.20944/preprints202504.0338.v1

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Direct PCR for Rapid and Safe Pathogen Detection: Field Testing in Emerging Infectious Disease Outbreaks?

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Abstract: Rapid, safe, and field-deployable molecular diagnostics are crucial for effective management of infectious disease outbreaks, particularly those involving highly infectious pathogens, which can produce clinical symptoms similar to **less infectious pathogens**, thus raising **potential** biosafety concerns. In this study, we evaluated DNA/RNA Defend Pro (DRDP) buffer, a novel viral-inactivating transport medium designed to stabilize nucleic acids and allow direct PCR without nucleic acid extraction. To ensure critical qPCR parameters were not compromised by using DRDP, we conducted serial dilution tests using herpes simplex viruses 1 and 2 (HSV-1, HSV-2) and varicella-zoster virus (VZV), comparing DRDP to standard universal transport medium (UTM). Detection sensitivity, determined by cycle quantification (Cq) values, favored DRDP, as UTM samples required a 2–3-fold dilution to mitigate PCR inhibition. DRDP maintained reliable PCR compatibility at reaction volumes containing up to 25% buffer. At higher DRDP concentrations (30–35%), PCR inhibition occurred due to EDTA content but was fully reversible by adding supplemental magnesium. Furthermore, DRDP samples did **not** require **an** initial 95 °C thermal lysis step, thus simplifying the procedure without reducing PCR sensitivity or efficiency.

Keywords: direct PCR; virus inactivation; point-of-care testing; outbreak diagnostics; nucleic acid stabilization

1. Introduction

Rapid detection of infectious agents is essential for controlling emerging infectious diseases [1]. Traditional PCR-based diagnostics typically require nucleic acid extraction prior to PCR, which introduces biosafety hazards, added complexity and costs, and delays in turnaround time [2]. Recent efforts have aimed to simplify diagnostic workflows by implementing direct PCR methods that omit extraction steps [3,4], thereby reducing time and resources required. However, the success of direct PCR depends on the medium used for sample transport, pathogen inactivation, and nucleic acid stabilization. Many standard transport media are not compatible with direct PCR due to the presence of PCR inhibitors or the need for complex sample handling [5,6].

DNA/RNA Defend Pro (DRDP) buffer has been developed as a universal, virus-inactivating transport medium formulated with chelating agents and mild detergents. It contains components including EDTA and citric acid (maintaining the buffer at approximately pH 2) along with a proprietary non-ionic detergent. This formulation ensures pathogen inactivation, compatibility with rapid antigen testing, and nucleic acid stability, without the PCR inhibitors commonly found in guanidinium-based lysis buffers [7]. Unlike guanidinium-based solutions – which strongly inhibit PCR and therefore require extraction [7] – DRDP buffer allows direct PCR on the sample without nucleic acid purification [8]. The buffer effectively lyses virions and preserves DNA/RNA, while also

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permitting downstream amplification. Moreover, by inactivating pathogens on contact, DRDP enhances biosafety for personnel handling clinical specimens [9].

An example of an emerging diagnostic challenge underscoring these needs is the recent global outbreak of mpox (formerly referred to as monkeypox) [10]. The vesiculopustular skin lesions caused by mpox can closely resemble those of herpesviruses such as VZV (chickenpox) or HSV, creating diagnostic uncertainty. Currently, swabs from suspected mpox lesions are typically placed in universal transport medium (UTM) or similar media that preserve virus viability during transport. While this allows traditional PCR testing in centralized laboratories, it poses biosafety risks – sample handling requires at least a Class II biosafety cabinet, and certain procedures (e.g., aliquoting) are recommended only in BSL-3 facilities [11]. An inactivating transport buffer like DRDP could mitigate these concerns by neutralizing the pathogen at the point of collection (rendering the sample noninfectious) and still preserving nucleic acids for direct PCR testing. This would enable safer and more efficient diagnostics for mpox in decentralized or resource-limited settings, where biosafety and rapid results are critical. Such an approach aligns with the broader need for point-of-care outbreak diagnostics, providing enhanced biosafety and timely results for improved outbreak response [12].

This study aimed to evaluate key aspects of DRDP's performance for direct PCR diagnostics using DNA viruses (HSV-1, HSV-2, and VZV) as a model. In particular, we examined: (a) detection sensitivity of DRDP vs. a standard medium (UTM) by comparing PCR Cq values across serial virus dilutions to determine whether DRDP compromises, maintains, or improves the limit of detection relative to the conventional medium; (b) PCR compatibility at high buffer concentrations, by varying the fraction of DRDP buffer in the reaction and assessing any amplification inhibition (and whether it can be mitigated by magnesium supplementation); and (c) the impact of thermal pre-treatment, evaluating if the usual 95 °C pre-heating lysis step is necessary when using DRDP, or if it can be omitted without affecting results. Additionally, we assessed the performance of DRDP in a commercial multiplex PCR assay to confirm its compatibility with established diagnostic platforms. By examining these factors, we provide a comprehensive evaluation of DRDP's utility for direct PCR in the field or point-of-care settings.

2. Materials and Methods

The **Sample Preparation and Virus Dilution:** HSV-1, HSV-2, and VZV stocks from anonymized leftover clinical samples were prepared at equivalent titers and used to inoculate transport media. Aliquots of each virus were serially diluted in 10-fold increments (10^0, 10^−1, 10^−2, etc.), into two different media: DRDP buffer (DNA/RNA Defend Pro™, InActiv Blue, Belgium), constituting 15-35% of total PCR reaction and a standard universal transport medium (UTM) as a control (15% of total PCR volume). The undiluted samples (1×) and each dilution were prepared in parallel for both DRDP and UTM. All samples were handled in a biosafety cabinet. Notably, DRDP-containing samples were rendered noninfectious immediately upon contact with the buffer, whereas UTM samples contained viable virus and were handled with appropriate precautions. No nucleic acid extraction was performed on any samples. DRDP samples were added directly into the PCR setup. The UTM samples were following 15 minutes thermal lysis (95°C) and 3x dilution, due to inhibitory effect of UTM on PCR [13].

PCR Assay and Conditions: The PCR assays targeted conserved regions of each virus's genome using RT-qPCR with a hydrolysis probe. *Table A1* (Appendix A) lists the primer and probe sequences specific for HSV-1, HSV-2, and VZV. The HSV-1 and HSV-2 assays target each virus's glycoprotein gene and the VZV assay targets the DNA polymerase gene. Primers and hydrolysis probes were synthesized by Integrated DNA Technologies (IDT, Coralville, IA, USA). Each viral target's probe was labeled with a distinct fluorophore to enable multiplex detection within a single reaction: FAM for HSV-1, HEX for HSV-2, and Cy5 for VZV.

Real-time PCR was performed using a Roche Light Cycler® 480 II instrument (Roche Diagnostics) with 20 µL reaction volume per sample. The thermal cycling program consisted of an initial 95 °C denaturation for 2 minutes, followed by 40 cycles of 95 °C for 15 seconds and 55 °C for

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30 seconds, with fluorescence acquisition in the appropriate channels during the 55 °C step. To test the effect of increasing buffer input, additional PCR reactions were prepared with the sample (in DRDP) constituting 15%, 20%, 25%, 30%, or 35% of the total reaction volume. Reactions exceeding 25% DRDP content were also run with 10mM $MgCl_2$ to counteract EDTA chelation.

Commercial Assay Evaluation: To assess compatibility with an existing diagnostic platform, we also evaluated DRDP-preserved samples on a commercial multiplex PCR assay. Aliquots of clinical swab samples known to be positive for HSV-1, HSV-2, or VZV were diluted in parallel into DRDP buffer (with 10 mM Mg^{2+} supplementation) and into standard UTM. Tests were done using the DiaSorin Simplexa™ HSV-1/2 & VZV Direct kit (DiaSorin, Italy) according to the manufacturer's instructions, where input sample (50 µL) and PCR reaction (50 µL) have the same volumes. The Cq results from the Simplexa assay for each sample were compared between DRDP and UTM collection conditions to determine any performance differences.

3. Results

3.1. Detection Sensitivity: DRDP vs. UTM

All three viruses were robustly detected in both media across all dilution levels, with DRDP performing equivalently to UTM regarding sensitivity. At the highest viral load (undiluted 1×), DRDP's Cq values were essentially the same as UTM's (differences ~0.5 cycles or less). As the samples became more diluted (10× and 100×), DRDP showed a slight advantage in detection. For example, HSV-1 at 1× yielded Cq 18.0 ± 0.1 in DRDP vs. 18.5 ± 0.2 in UTM, and VZV at 10× gave Cq 23.0 ± 0.4 in DRDP vs. 24.0 ± 0.2 in UTM. At the 100× dilution (lower viral copies), DRDP samples had Cq values about 1.0–1.2 cycles lower (earlier, more sensitive) than the corresponding UTM samples for all three viruses. For instance, HSV-2 at 100× showed a mean Cq of 25.9 ± 0.5 in DRDP vs. 27.1 ± 0.6 in UTM. Table 2 summarizes the mean Cq results (±SD) for each virus at three dilution levels in each medium. Even at a 100-fold dilution without extraction or concentration, viruses in DRDP were still reliably detected with Cq values in the mid-20s, comparable to those in the conventional medium. This indicates that DRDP did not compromise sensitivity and may even slightly improve the detection of low-abundance targets, possibly through more effective lysis of virions or better preservation of nucleic acid integrity.

Table 1. PCR cycle quantification (Cq) values for HSV-1, HSV-2, and VZV in samples collected in UTM vs. DRDP buffer at various dilution factors. Values are mean Cq ± SD (n = 3) for each condition. Each PCR reaction mix received the same equivalent sample volume.

dilution	medium	HSV-1 Cq	HSV-2 Cq	VZV Cq
1x	UTM	18.5 ± 0.2	19.2 ± 0.3	21.0 ± 0.3
1x	DRDP	18.0 ± 0.1	18.7 ± 0.2	20.5 ± 0.2
10x	UTM	22.0 ± 0.3	23.0 ± 0.4	24.0 ± 0.2
10x	DRDP	21.8 ± 0.2	22.5 ± 0.3	23.0 ± 0.4
100x	UTM	25.5 ± 0.5	27.1 ± 0.6	27.9 ± 0.4
100x	DRDP	24.3 ± 0.4	25.9 ± 0.5	26.7 ± 0.6

3.2. Effect of DRDP Buffer Volume on PCR Inhibition

We next examined whether increasing the proportion of DRDP buffer in the PCR reaction would introduce any inhibition of amplification. Since DRDP contains EDTA (which chelates divalent cations including Mg^{2+}) and is acidic, using high concentrations could sequester magnesium or otherwise alter the reaction environment. A series of reactions were run where the fraction of the PCR volume composed of DRDP (with sample) ranged from 10% up to 35%, using undiluted HSV-1 as the test system. Table 2 summarizes the observed inhibition and Cq shifts. Reactions with up to 25% of the total volume as DRDP buffer showed no detectable inhibition. Amplification curves in these reactions were virtually identical to those of control reactions with smaller DRDP inputs, and Cq

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values shifted by no more than 0.5 cycles (Table 2). At 30% DRDP, a mild delay in amplification was observed (Cq shifts of ~1–2 cycles), and at 35% DRDP, significant inhibition occurred (markedly delayed or failed amplification). As described in the manufacturer's manual, inhibition at high DRDP concentration might be due to EDTA in the buffer chelating magnesium necessary for PCR. To test this, additional Mg^{2+} was added to reactions with 35% DRDP. With magnesium supplementation, the 35% DRDP reactions showed no notable inhibition. Cq values returned close to baseline and amplification curves regained a normal sigmoidal shape. This "rescue" demonstrates that inhibition at high DRDP volumes was indeed due to magnesium sequestration and not due to irreversible enzyme inhibition or nucleic acid damage from the buffer.

Table 2. Effect of DRDP buffer fraction and Mg^{2+} supplementation on PCR amplification. "PCR inhibition" qualitatively describes the outcome, and "Cq shift" quantifies the delay in Cq compared to a control reaction with a low DRDP fraction (≤25%). The n.d. term is used for a reaction which was not done.

DRDP in PCR	inhibition (no extra Mg^{2+})	Cq shift	inhibition (added extra Mg^{2+})
15%	absent	<1	n.d.
20%	absent	<1	n.d.
25%	absent	<1	n.d.
30%	present	<=2	none
35%	present	failed	none

Additionally, the efficacy of PCR reaction is preserved for various qPCR tests having 15-25% DRDP input pf PCR reaction volume, as demonstrated in Appendix Table A2.

3.3. Omission of Initial Heat Lysis Step

Another finding of our study was that initial high-temperature lysis of sample material before the start of PCR protocols may not be necessary for samples in DRDP. Conventionally, a 15 minute at 95 °C incubation is used to ensure complete sample lysis, by so-called "thermal lysis". However, DRDP's formulation lyses viral particles (due to its acidic, detergent-containing nature). We hypothesized that this might render the extended heat step of 15 minutes redundant. To test this, we performed PCR on DRDP-preserved samples with and without the initial 95 °C, 15 minutes hold.

The results showed no significant difference in amplification performance whether or not the 95 °C pre-treatment was applied. When the thermal pre-step was omitted (samples remained at room temperature during that period and then entered standard cycling program), the Cq values obtained were virtually identical to those with the 15 minutes hold heat step – differences were <0.3 cycles on average, well within run-to-run variability. Amplification curves for both conditions were superimposable, indicating that the targets were equally available. There was no significant Cq delay, or loss of fluorescence intensity observed among these two groups. In practical terms, this means DRDP-treated samples were "PCR-ready" without a prolonged initial thermal lysis step. The potent chemical lysis and nucleic acid release achieved by DRDP at room temperature appeared sufficient to make the viral DNA accessible. As soon as thermal cycling began, the brief denaturation during the first cycle (95 °C for 120 s) was enough to denature the template and activate the polymerase.

3.4. Performance in a Commercial PCR Assay

In a set of paired clinical swab samples tested on the DiaSorin Simplexa HSV-1/2 & VZV Direct PCR platform, DRDP collection performed comparably to UTM. All samples that were positive when collected in UTM were also positive with DRDP supplemented with 10 mM Mg 2+, with Cq values that were equal or slightly lower (earlier) in the DRDP-preserved samples. On average, samples collected in DRDP showed ~1 Cq units lower than those collected in UTM on the Simplexa assay, indicating a small increase in analytical sensitivity. This demonstrates that DRDP is compatible with

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existing commercial diagnostic assays, providing equivalent results while also offering the biosafety advantage of immediate pathogen inactivation at the point of collection.

4. Discussion

This study provides original data on the performance of the DNA/RNA Defend Pro (DRDP) buffer in direct PCR applications, using HSV-1, HSV-2, and VZV as model DNA viruses. Our results demonstrate that DRDP offers significant advantages compared to a traditional medium (UTM) by maintaining nucleic acid stability, enhancing PCR sensitivity, and ensuring virus inactivation simultaneously. Notably, DRDP enabled direct amplification without any extraction or purification steps, with no loss of sensitivity relative to the direct PCR methods using UTM medium. In fact, DRDP showed a tendency toward slightly lower Cq values, suggesting it may even improve the detection of low-abundance targets – likely through more efficient lysis of virions, absence of inhibition and protection of DNA from degradation [14].

These findings are consistent with prior evaluations of DRDP and similar inactivating media in other pathogen systems [8,9]. For example, recent studies on SARS-CoV-2, influenza, and RSV have demonstrated that using an inactivating transport medium like DRDP can support direct PCR without compromising sensitivity. Our work extends those observations to herpesviruses and demonstrates the broad applicability of an inactivation-based direct PCR approach for DNA virus detection. Importantly, the inherent pathogen inactivation provided by DRDP substantially enhances biosafety during sample handling, as infectious agents are neutralized at the point of collection. This addresses a major safety concern of direct PCR methods, which otherwise might require handling of live pathogen. By combining inactivation and stabilization in one step, DRDP serves as both a transport medium and a PCR-ready lysis buffer. The implications of our results are significant for outbreak response and point-of-care testing. DRDP buffer allows clinicians or field workers to collect swab samples and run PCR on-site without need for a biosafety hood for extraction or complex reagent kits. The sample can be added directly to a PCR mix and run on a portable thermocycler. The time savings (both from skipping extraction and/or thermal lysis) can improve patient management and infection control measures. The confirmed stability of nucleic acids in DRDP ensures that samples remain suitable for testing even in challenging field conditions [8,9]. Similar extraction-free molecular testing approaches have been successfully implemented for SARS-CoV-2 during the COVID-19 pandemic, demonstrating the feasibility of deploying rapid PCR diagnostics in non-traditional settings [15–18].

An additional operational advantage of DRDP is its ability to facilitate the preparation of biologically relevant whole-process positive and negative controls that retain 8 days stability at +25° C during weekly operations. Traditionally, laboratories must store such controls at −20 °C or lower to prevent nucleic acid degradation, often requiring extensive aliquoting into single-use volumes to avoid repeated freeze–thaw cycles that accelerate molecular decay and reduce assay reliability. This approach consumes considerable freezer space and increases preparation workload. In contrast, DRDP chemically stabilizes nucleic acids by combining acidification and EDTA chelation, enabling ambient temperature storage of control materials for extended durations without measurable degradation—even under repeated temperature fluctuations associated with field or routine diagnostic use [8,9,13,19]. This eliminates the need for frozen stocks or guanidinium-based buffers, the latter of which are incompatible with direct PCR due to strong enzyme inhibition [7]. Whole-process positive controls prepared in DRDP, such as swabs spiked with inactivated pathogen and/or characterized patient material, can be stored as ready-to-use aliquots at room temperature or 4 °C, offering unmatched convenience and reliability. These DRDP-preserved controls undergo the same steps as patient samples—including swabbing, elution, and amplification—thus ensuring a realistic, end-to-end quality control. The ability to prepare large batches of stable aliquots simplifies workflow logistics and mitigates the risk of degradation from repeated freezing, thawing, or handling errors. Importantly, DRDP also supports the preparation of biologically complex negative controls—e.g., pooled swab specimens from known-negative individuals or matrices such as mucosal samples—

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that mimic real samples in composition but lack the target pathogen. These "whole process" negative controls can be carried out through the full workflow to confirm specificity, assess cross-contamination, and detect non-specific amplification. As molecular testing expands to decentralized settings, including mobile units and remote clinics, the ability to deploy ambient-stable, whole-process controls becomes increasingly valuable. The operational benefits include reduced cold-chain dependency, minimized preparation burden, and consistent test validation under real-use conditions. These properties make DRDP-based controls especially suitable for field-deployable diagnostics and for maintaining quality assurance in outbreak response programs.

5. Conclusions

Direct PCR enabled by the DRDP buffer offers a rapid and safe diagnostic approach for infectious diseases, eliminating the need for time-consuming extraction step maintaining sensitivity. Our evaluation with HSV-1, HSV-2, and VZV demonstrates that DRDP-preserved samples can be directly amplified with performance comparable to conventional methods, even improving detection of low-level targets. The buffer's inactivation capability addresses biosafety concerns, and its compatibility with PCR simplifies workflows in outbreak and point-of-care settings. By reducing processing steps and equipment requirements, DRDP-facilitated direct PCR can accelerate diagnosis and help contain outbreaks more effectively. Ongoing and future studies extending this approach to other pathogens (including RNA viruses) and integrating it with field-deployable PCR platforms will further validate DRDP's role in advancing molecular diagnostics while also protecting laboratory workers from exposure to potential pathogens.

Although rapid antigen testing (RAT) was not evaluated in the current study, implementing RAT as a preliminary screening method could provide rapid, cost-effective identification of positive cases, while subsequent molecular nucleic acid amplification testing (NAT) of RAT-negative samples would ensure enhanced diagnostic accuracy and overall reliability, potentially optimizing resource allocation and improving testing efficiency.

6. Patents

The composition of DRDP medium is the intellectual property of InActiv Blue.

Author Contributions: Conceptualizations and work execution are shared among I.B. and M.O.

Funding: This research received no external funding.

Institutional Review Board Statement and Informed Consent Statement: As per our hospital institutional review board (IRB) policies, this project was a laboratory quality improvement program and thus, further IRB approval was not required.

Data Availability Statement: All relevant data are present in the Result section of this manuscript.

Acknowledgments: The authors thank Dr. Jo Vandesompele, Dennis Berteloot, and other developers of DNA/RNA Defend Pro (InActiv Blue) for providing DRDP medium free of charge. We also extend our appreciation to the DiaSorin Simplexa R&D team, including Dr. Michelle Tabb, and colleagues, for their support in validations and insights during the evaluation process. Special thanks to Coleen Delisle (CCOMTL) from the Molecular Microbiology Unit at OPTILAB MUHC for providing real-life clinical samples critical to this study. We are grateful to Drs. Marc-Christian Domingo, André Dascal, and Fabien Rallu (Montreal, QC) for valuable early discussions on the importance of direct PCR in rapid diagnostic screening.

Conflicts of Interest: The authors declare no conflicts of interest.

 doi:10.20944/preprints202504.0338.v1

Abbreviations

The following abbreviations are used in this manuscript:

UTM	Universal Transportation Medium
DRDP	DNA/RNA Defend Pro
PCR	Polymerase Chain Reaction
mpox	monkey pox virus

Appendix A

Table A1. Oligonucleotide primers and probes used for real-time PCR detection of HSV-1, HSV-2, and VZV.

virus	forward primer (5′→3′)	reverse primer (5′→3′)	probe (5′→3′)
HSV-1	CGGCCGTGTGACACTATCG	CTCGTAAAATGGCCCCTCC	CCATACCGACCACACCGACGAACC
HSV-2	CGCCAAATACGCCTTAGCA	GAAGGTTCTTCCCGCGAAAT	CTCGCTTAAGATGGCCGATCCCAATC
VZV	CACGTATTTTCAGTCCTCTTCAAGTG	TTAGACGTGGAGTTGACATCGTTT	TACCGCCCGTGGAGCGCG

Table A2. PCR amplification efficiency at various DRDP buffer fractions. Each slope was determined from the standard curve of Cq values across a 10^0, 10^(-1),10^(-2) and 10^(-3) dilution series of VZV-positive clinical sample.

DRDP buffer in reaction (%)	standard curve slope	calculated efficiency (%)
15%	-3.3	100.5
20%	-3.1	108.8
25%	-3.2	101.4

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