PCR*opsis*™ Reagent RVD-RT Outperforms Promega XpressAmp™ for direct PCR applications at Room Temperature

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Abstract:
Direct PCR, where the user does not perform RNA or DNA extraction before PCR, holds great promise for the PCR molecular diagnostics industry. Such an approach saves time and money while streamlining protocols and potentially reducing errors. However, many direct PCR technologies fail to compare favorably with traditional RNA and DNA extraction. This has impeded the widespread adoption of direct PCR technologies in many clinical laboratories.

In this report, we compare Entopsis PCR*opsis*™ Reagent RVD-RT to Promega XpressAmp™ Direct Amplification Reagents to mediate direct RT-qPCR from SARS-CoV-2 viral samples in various transport mediums and saliva at room temperature. The PCR*opsis*™ direct PCR product, unlike the products from Promega, resulted in cycle thresholds (Ct) comparable to RNA extraction from viral samples in most transport mediums and saliva. Moreover, the direct PCR product from Promega often resulted in no cycle thresholds for viral samples in various transport mediums. The low sensitivity of Promega direct amplification would have resulted in several false negative readings and failed to detect viral infected samples in the clinic. PCR*opsis*™ Reagent RVD-RT offers a reliable and universal direct PCR solution for viral samples shipped in various transport mediums as well as in saliva without sacrificing assay sensitivity.

Results:

	N1		N2		E		N (Japan)	
	Reagent RVD-RT	Promega XpressAmp	Reagent RVD-RT	Promega XpressAmp	Reagent RVD-RT	Promega XpressAmp	Reagent RVD-RT	Promega XpressAmp
Extracted RNA	**28.74**		**34.74**		**28.75**		**39.79**	
BD UVT	27.19	28.61	28.66	34.44	28.55	37.23	34.16	44.87
WHO-VTM	28.2	30.21	36.59	None	28.9	None	36.69	None
CDC-VTM	28.97	28.37	30.95	32.84	28.94	44.55	39.55	44.92
MedSchenker VTM	29.66	30	28.83	37.37	27.93	37.63	32.34	43.45
SORFA VTM	31.8	28.78	30.84	34.95	30.94	43.92	39.86	40.64
Han Chang UTM	31.68	28.62	30.46	None	31.87	40.64	39.94	35.42
Bartels VTM	28.94	27.08	37.13	None	31.17	35.72		
Noble Biosciences CTM	32.39	29.5	30.06	37.48	30.64	43.2	40.2	44.35
NEST VTM	30.5	30.04	30.73	37.43	30.31	41.04	40.62	None
PBS	28.52	37.42	29.35	None	28.99	None	37.74	41.22
Citoswab VTM	28.76	36.19	29.24	None	29.61	None	37.68	None

Table 1. RT-qPCR Cycle Thresholds for Direct PCR Technologies with SARS-CoV-2 at Multiple Gene Targets in Various Transport Mediums. Direct PCR mediated by Reagent RVD-RT resulted in Ct readings from various gene regions comparable to traditional RNA extraction for tested transport mediums. In contrast, direct PCR mediated by Promega XpressAmp™ usually resulted in 4~11+ Ct readings compared to the RNA extraction control and failed to produce any Ct readings for various transport mediums. Each sample was tested in duplicates and the indicated Ct is the average of two readings. Cells in red indicate Ct readings 4+ compared to those of Reagent RVD-RT.

	N1	N2	RP	E
Extracted RNA	27.52	34.44	40.11	28.83
Reagent RVD-RT + RVD Enhancer	26.78	29.72	25.48	29.11
Promega XpressAmp	30.47	41.23	37.26	36.53

Table 2. RT-qPCR Cycle Threshold readings for Direct PCR Technologies with SARS-CoV-2 in Saliva. Direct PCR mediated by Reagent RVD-RT resulted in comparable or lower Ct at various gene targets when compared to traditional RNA extraction for tested saliva samples. In contrast, direct PCR mediated by Promega XpressAmp™ resulted in lower sensitivity, with 3~11 Ct readings greater compared to the RNA extraction control and Reagent RVD-RT. Each sample was tested in duplicates and the indicated Ct is the average of two readings. Cells in red indicate Ct readings >4+ compared to those of Reagent RVD-RT.

Key Conclusions:

- Reagent RVD-RT significantly outperforms Promega XpressAmp™ Direct Amplification Reagents in 3 key areas:
    - samples tested with SARS-CoV-2 in diverse transport mediums
    - samples tested with SARS-CoV-2 in human saliva
    - samples tested at different gene regions

- The data indicates a false-negative rate of 0% if Reagent RVD-RT is substituted for traditional RNA extraction for COVID-19 testing when examining N1 / N2 gene targets.
    - In contrast, the use of Promega XpressAmp™ Direct Amplification Reagents as an alternative to traditional RNA extraction would have resulted in 45% of positive samples being called 'indeterminate'.

- Reagent RVD-RT resulted in Ct readings comparable (+/- 3 Ct) to RNA extraction in almost all tested configurations.
    - In contrast, Promega XpressAmp™ Direct Amplification Reagents usually resulted in Ct readings over 5 compared to the RNA extraction controls or failed to produce readings for various configurations.

- In conclusion, Reagent RVD-RT can substitute traditional RNA extraction for the identification of viruses through RT-qPCR from specimens in transport medium and saliva.

Entopsis' PCR*opsis*™ brand of direct PCR products utilize a unique set of proprietary nanotechnologies. This novel approach likely accounts for the observed improvement in functionality over traditional approaches like Promega's XpressAmp™ and other direct PCR technologies (data not shown), while offering results that are comparable to nucleic acid extraction. As such, Entopsis' PCR*opsis*™ brand of direct PCR products should be viewed as distinct from traditional direct PCR approaches using outdated technologies that offer suboptimal performance.

Methods:

Materials:

- Heat inactivated remnant clinical samples
- Promega: 1-Step GoTaq® RT-qPCR Systems
- IDT: SARS-CoV-2 (2019-nCoV) CDC qPCR Probe Assay
- Universal Viral Transport (UVT)
- Viral Transport Medium (VTM)
- Clinical Transport Medium (CTM)
- Entopsis: PCR*opsis*™ Reagent RVD-RT (with PCR*opsis*™ Activator)
- Promega: XpressAmp™ Direct Amplification Reagents
- Stellar Scientific: Thin-walled PCR tubes
- Chai: Open qPCR Thermocycler

Prepare viral samples in transport mediums or saliva:

1. Dilute 1 volume heat inactivated remnant clinical samples with 9 volumes of transport medium or saliva
2. Mix thoroughly

Studies with PCR*opsis*™ Reagent RVD-RT:

1. Thoroughly mix 950 µL Reagent RVD-RT with 50 µL Activator
    a. Referred to as just Reagent RVD-RT
2. Thoroughly mix 20 µL Reagent RVD-RT with 20 µL of diluted viral samples in a thin walled tube (0.2 ~ 0.6 mL) and cap tubes
3. Incubate Reagent RVD-RT mixture with viral sample for 10 minutes at 25˚C
4. Add 5 µL of Reagent RVD-RT with viral sample to 15 µL of RT-qPCR mix.
    a. RT-qPCR mixture:
        i. Promega GoTaq® qPCR Master Mix, 2X: 10 µl
        ii. Promega 1X GoScript™ RT Mix for 1-Step RT-qPCR (50X): 0.4 µl
        iii. IDT primer / probe: 1.5 µl
        iv. Nuclease-Free Water: 3.1 µl
        v. Reagent RVD-RT / viral sample: 5 µL
5. Run samples on qPCR Thermocycler for 45 cycles.
    a. Reverse Transcription: 45˚C for 15 minutes, then 95˚C for 2 minutes
    b. DNA Amplification: 95˚C 30 seconds, 55˚C 30 seconds for 45 cycles
    c. Extension: 55˚C 60 seconds
    d. Hold: 4˚C

Studies with Promega XpressAmp™ Direct Amplification Reagents:

1. Add 1-Thioglycerol to a concentration of 1% (v/v) to an aliquot of XpressAmp™ Lysis Buffer (called XpressAmp Mixture)
    a. Use within 1 hour of preparation
2. Thoroughly mix 20 µL XpressAmp Mixture with 20 µL of diluted viral samples in a thin walled tube (0.2 ~ 0.6 mL) and cap tubes
3. Incubate XpressAmp Mixture with viral sample for 10 minutes at 25˚C
4. Mix thoroughly.
5. Add 5 µL of heated XpressAmp Mixture with viral sample to 15 µL of RT-qPCR mix.
    a. RT-qPCR mixture:
        i. Promega GoTaq® qPCR Master Mix, 2X: 10 µl
        ii. Promega 1X GoScript™ RT Mix for 1-Step RT-qPCR (50X): 0.4 µl
        iii. IDT primer / probe: 1.5 µl
        iv. XpressAmp™ Solution: 4 µl
        v. XpressAmp Mixture / viral sample: 5 µL
6. Run samples on qPCR Thermocycler for 45 cycles.
    a. Reverse Transcription: 45˚C for 15 minutes, then 95˚C for 2 minutes
    b. DNA Amplification: 95˚C 30 seconds, 55˚C 30 seconds for 45 cycles
    c. Extension: 55˚C 60 seconds
    d. Hold: 4˚C

Viral RNA Extraction:

Follow manufacture's protocol: Qiagen QIAamp Viral RNA Kit