Clinical Prospective Study comparing In-house Respiratory Viral Assays With a Highly Multiplexed Commercial Assay developed by SpeeDx

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Introduction

Multiplex qPCR is often used for the simultaneous detection of respiratory viruses in clinical specimens. Economic and seasonally-driven pressures for faster, accurate sample testing at reduced costs have resulted in an increased need for highly sensitive and specific assays with high throughput for respiratory illnesses. The PlexPCR™ RV 11 (beta) assay (SpeeDx, Australia) detects 11 virus-specific targets in a 2-well format and utilises a novel qPCR technology that provides a powerful tool for multiplexing(1). Here we evaluated this platform in comparison with an in-house 10-virus targeted multiplex PCR assay which detects a broad range of respiratory viruses(2).

1 Mokany et al JACS (2010) & Clin Chem (2013); 2 Ratnamohan et al Vir J (2014)

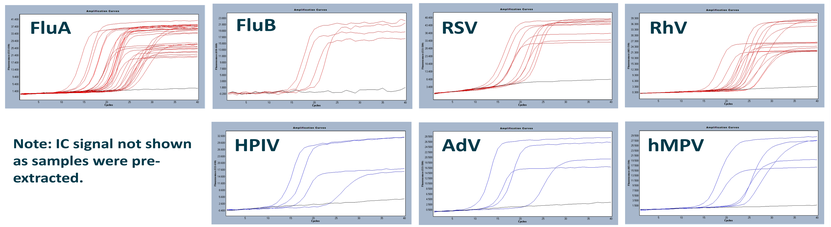
Assay comparison

Well	In-house	PlexPCR™ RV11
1	Flu A, Flu B, RSV A&B	Flu A, Flu B, RSV A&B, RhV , Internal Control
2	HPIV 1-3	HPIV 1-4, hMPV, AdV B&C
3	RhV, EV, hMPV	
4	AdV, Internal Control	

Flu A : Influenza A
Flu B : Influenza B
RhV : Rhinovirus
RSV A&B: Respiratory Synctial Virus A&B
HPIV 1-4: Human Parainfluenza 1-4
hMPV : Human Metapneumovirus
AdV B&C: Adenovirus B&C

Improved workflow with 2 well assay

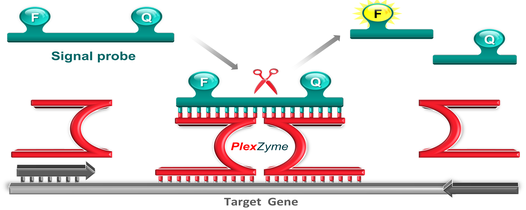
Clinical Evaluation Amplification Curves



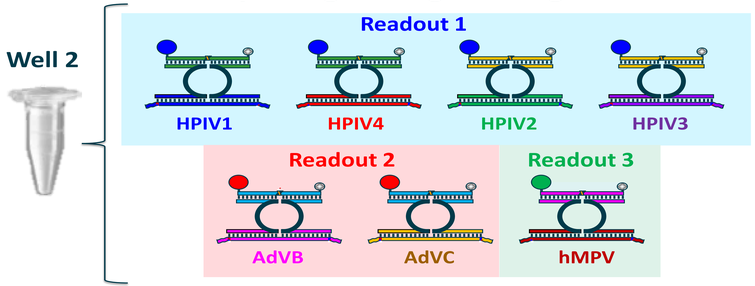
Note: IC signal not shown as samples were pre-extracted.

Efficient amplification curves seen in highly multiplexed assay

PlexPCR™ utilising novel PlexZyme™ detection



Flexible multiplexing strategies using PlexPCR™



Superior Multiplexing Capacity, Highly Specific & Sensitive

PlexPCR™ vs Westmead In-house Assay

FluA

SpeeDx	In-house qPCR +	In-house qPCR -	Total
+	26	7	33
-	0	171	171
Total	26	178	204

AdV

SpeeDx	In-house qPCR +	In-house qPCR -	Total
+	3	3	6
-	0	198	198
Total	3	201	204

FluB

SpeeDx	In-house qPCR +	In-house qPCR -	Total
+	4	0	4
-	0	200	200
Total	4	200	204

RhV

SpeeDx	In-house qPCR +	In-house qPCR -	Total
+	9	12	21
-	1	182	183
Total	10	194	204

HPIV

SpeeDx	In-house qPCR +	In-house qPCR -	Total
+	3	1	4
-	0	200	200
Total	3	201	204

RSV

SpeeDx	In-house qPCR +	In-house qPCR -	Total
+	10	0	10
-	0	194	194
Total	10	194	204

hMPV

SpeeDx	In-house qPCR +	In-house qPCR -	Total
+	1	5	6
-	0	198	198
Total	1	203	204

FluB & RSV results were 100% concordant

No. of clinical samples: 204

Sample type: upper respiratory tract & nasopharyngeal swab tested over 3 days

Rapid & Robust Performance with Good Clinical Specificity & Sensitivity

Resolved Discrepant samples

Target	Discrepant	Resolution for PlexPCR	Method	Sensitivity (%)	Specificity (%)
FluA	7 positives	5 true positive; 2 nt*	GeneXpert	100.0	100.0
RhV	12 positives 1 negative	10 true positive; 1 false positive 1 confirmed negative; 1 nt*	Sequencing	100.0	98.9
hMPV	5 positives	2 confirmed; 3 to be resolved	Sequencing	100.0	97.5
AdV B&C	3 positives	To be resolved	Sequencing	100.0	98.5
HPIV1-4	1 positive	To be resolved	Sequencing	100.0	99.5

* nt – not tested, sample unavailable

Conclusion

The PlexPCR™ RV 11 (beta) assay demonstrated high sensitivity and specificity even in complex multiplex assays with decreased reaction setup from 4 to 2 wells. We found the PlexPCR assay provided a good approach to qPCR with the advantages of a robust performance in multiplex. There are also advantages seen in higher throughput which would have additional cost savings.

SpeeDx

***Plex**PCR™* **is a flexible, rapid & cost-effective technology for multiplexed detection of targets and genetic variants**

If you are interested in multiplexing your assay and/or wanting to achieve specific single base discrimination contact **info@speedx.com.au** or for more information about ***Plex**Prime™* & ***Plex**PCR™* technology visit **www.speedx.com.au**