

# **Comparison of Quidel Lyra Influenza A+B and RSV+hMPV to the Altona RealStar Influenza and RSV RT-PCR assays on the Applied Biosystems 7500 Fast Dx PCR System in a high volume laboratory setting**

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# Disclosure

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- For the current evaluation kits were supplied by Quidel Corporation [San Diego, CA] and Altona Diagnostics, [Hamburg, Germany]
- Instrumentation and consumables were supplied by Life Technologies [Carlsbad, CA]
- No other financial support was received in order to carry out this evaluation

# Introduction

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- In many centers, molecular methods have become the standard for the diagnosis of respiratory viruses particularly Influenza A/B and RSV
  - Most centers are using Real-Time PCR assays
  - Specimens include NP swabs/aspirates, BAL samples and less commonly sputum, throat swabs, nasal swabs and lung tissue
- Assays may differ in extraction steps required, targets amplified/detected, instrumentation used and time to results as well as in multiplexing
- Some have suggested that the sensitivity of molecular assays may decrease when targets are combined in multiplex assays versus individual target assays

# Purpose

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- To evaluate the sensitivity/specificity, ease of use, and time to results for 2 real-time PCR assays for the detection of Influenza A/B and RSV:
  - RealStar Influenza A/B and RSV assays (Altona Diagnostics, Hamburg, Germany)
  - Lyra Influenza A/B & RSV/hMPV assays (Quidel Diagnostics, San Diego, CA, USA)

# Methods

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- Specimens:
  - 401 consecutive respiratory samples (BAL and NP swabs) from October 2012 to July 2013
- Testing performed as per manufacturer's kit insert
- Results of the RealStar assays and Lyra assays were compared with those of the Luminex RVP xTAG Fast assay (Austin, TX)
- Fresh samples were initially tested on the RVP xTAG Fast assay and retrospective samples were re-tested on the RealStar and Lyra assays
- Discrepant results between the different assays were aligned and 2 out of 3 concordant results was used to define a “true positive” and “true negative” result

# RealStar Influenza A/B and RSV Assays

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**EasyMag**



**Rotorgene**

# Lyra Flu A/B & RSV/hMPV Assays

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**EasyMag**



**ABI 7500 Fast Dx  
PCR System**

# Results

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Influenza A		Luminex		
		Positive	Negative	Total
Real-Star	Positive	33	7	40
	Negative	1	337	338
	Total	34	344	378

Overall concordance = 97.9% (370/378)



# Results

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<b>Influenza B</b>		<b>Luminex</b>		
		<b>Positive</b>	<b>Negative</b>	<b>Total</b>
<b>Real-Star</b>	<b>Positive</b>	13	3	16
	<b>Negative</b>	0	362	362
	<b>Total</b>	13	365	378

Overall concordance = 99.2% (365/378)

# Results

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<b>Influenza A</b>		<b>Luminex</b>		
		<b>Positive</b>	<b>Negative</b>	<b>Total</b>
<b>Lyra</b>	<b>Positive</b>	31	0	31
	<b>Negative</b>	3	367	370
	<b>Total</b>	34	367	401

Overall concordance = 99.3% (398/401)

# Results

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<b>Influenza B</b>		<b>Luminex</b>		
		<b>Positive</b>	<b>Negative</b>	<b>Total</b>
<b>Lyra</b>	<b>Positive</b>	11	0	11
	<b>Negative</b>	2	388	390
	<b>Total</b>	13	388	401

Overall concordance = 99.3% (399/401)

# Results

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	Flu A (%; 95% CI)		Flu B (%; 95% CI)	
	RealStar	Lyra	RealStar	Lyra
<b>Sensitivity</b>	97 (85-99)	91 (77-97)	100 (77-100)	85 (58-96)
<b>Specificity</b>	98 (96-99)	100 (99-100)	99 (98-100)	100 (99-100)
<b>PPV</b>	83 (68-91)	100 (89-100)	81 (57-93)	100 (74-100)
<b>NPV</b>	100 (98-100)	99 (98-100)	100 (99-100)	99 (98-100)
<b>Prevalence</b>	9 (7-12)	8 (6-12)	3 (2-6)	3 (2-5)
<b>% Agreement</b>	98	99	99	100

# Discordant Results: Lyra vs Luminex

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Sample #	Luminex	Lyra	RealStar
1	Flu A	Neg	Flu A
2	Flu A (H1N1)	Neg	Flu A
3	hMPV	Neg	N/A
4	RSV	Neg	RSV A
5	hMPV	Neg	N/A
6	Flu A (H1N1)	Neg	Neg
7	hMPV	Neg	N/A
8	Flu B	Invalid	Flu B
9	hMPV	Neg/Invalid	N/A
10	Flu B	Neg	Flu B

# Results

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RSV		Luminex		
		Positive	Negative	Total
Real-Star	Positive	15	9	24
	Negative	0	354	354
	Total	15	363	378

Overall concordance = 97.6% (369/378)

# Results

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RSV		Luminex		
		Positive	Negative	Total
Lyra	Positive	14	0	14
	Negative	1	386	387
	Total	15	386	401

Overall concordance = 99.9% (400/401)

# Results

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	RSV (%; 95% CI)	
	RealStar	Lyra
<b>Sensitivity</b>	100 (80-100)	93 (70-99)
<b>Specificity</b>	98 (95-99)	100 (99-100)
<b>PPV</b>	63 (43-79)	100 (78-100)
<b>NPV</b>	100 (99-100)	100 (99-100)
<b>Prevalence</b>	4 (2-6)	4 (2-6)
<b>% Agreement</b>	98	100



# Discordant Results: RealStar vs Luminex

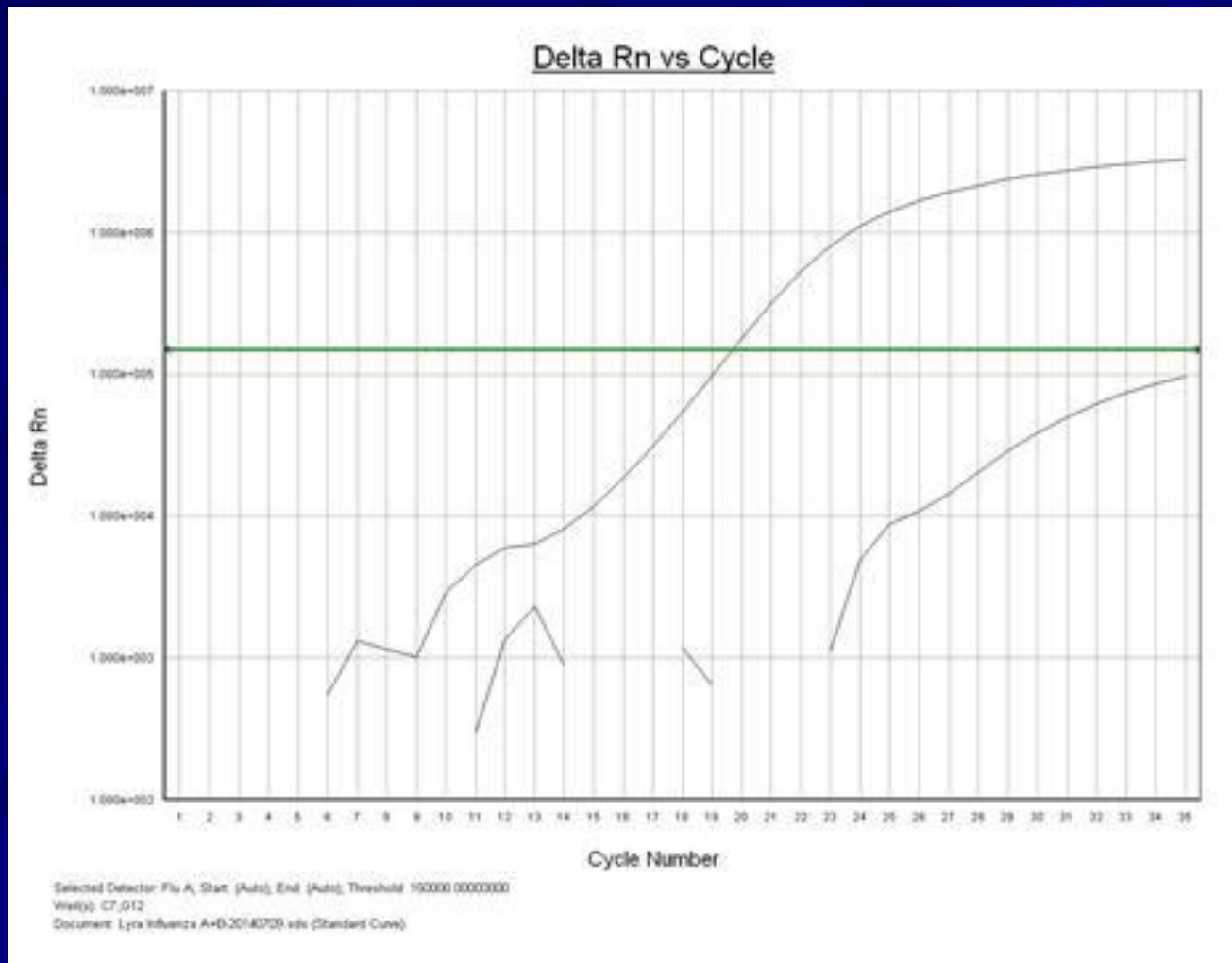
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Sample #	Luminex	RealStar	Lyra
1	Corona NL63	RSV	Neg
2	Corona HKU1	RSV	Neg
3	Corona HKU1	RSV	Neg
4	Corona 229E	RSV	Neg
5	Corona HKU1	RSV	Neg

# All Discordant Results:

Sample ID	Luminex Results	Altona Results	Quidel Results
ICS 1076	Flu A	Flu A	Neg
iCS 1300002	Corona HKU1	Flu A	Neg
ICS 1300013	Enterovirus/Rhinovirus	Flu B	Neg
ICS 1300019	Neg.	Flu B	Neg
ICS 1300021	Corona OC 43	Flu A	Neg
ICS 1300030	Corona NL63	RSV A	Neg
ICS 1300032	Neg.	Flu A	Neg
ICS 1300039	Neg.	RSV B	Neg
ICS 1300041	Enterovirus/Rhinovirus	Flu A	Neg
ICS 1300080	Enterovirus/Rhinovirus	RSV B	Neg
ICS 1300231	Corona HKU1	RSVA/B	Neg
ICS 1300304	Corona HKU1	RSV A	Neg
ICS 1300358	Neg.	Flu A	Neg
ICS 1300370	Neg.	RSV B	Neg
ICS 1300385	Corona 229E	RSV A/B	Neg
ICS 1300387	Flu A (H1N1)	Flu A	Neg
ICS 1300391	Corona HKU1	Flu A	Neg
ICS 1300398	Corona HKU1	RSV B	Neg
ICS 1300407	Metapneumovirus	N/A	Neg
ICS 1300476	RSV	RSV A	Neg
ICS 1300542	Metapneumovirus	N/A	Neg
ICS 1300553	Flu A (H1N1)	Neg	Neg
ICS 1300556	Metapneumovirus	N/A	Neg
ICS 1300582	Flu B	Flu B	Invalid
ICS 1300646	Flu B	RSV A / Flu B	Flu B 28.7
ICS 1300656	Metapneumovirus	N/A	Neg
ICS 1300667	Flu B	Flu B	Neg
ICS 1300692	Neg.	Flu A	Neg
ICS 1300710	Para 3	Flu B	Neg

# Discordant Result: Flu A



# Discordant Result: Flu B



# Results

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hMPV		Luminex		
		Positive	Negative	Total
Lyra	Positive	14	0	14
	Negative	4	383	387
	Total	18	383	401

Overall concordance = 99.0% (397/401)

# Discordant Result: hMPV



# Time to Results

## Elapsed time



# Time to Results

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- Results available:
  - RealStar:
    - 225 minutes (3.75 hours)
    - Up to 65 results
    - Tested in batch mode
  - Lyra:
    - 170 minutes (2.83 hours)
    - Up to 93 results
    - Tested in batch mode



# Summary

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- Overall no difference detected in sensitivity/specificity of assays; slight trend towards increased RSV detection using the assay RealStar, but decrease in PPV
- Discordant analysis with the Luminex assay suggests:
  - RealStar tends to give more positives (only 1 neg)
  - Lyra gives more negatives (No positives)
- Results available faster with Lyra assay than RealStar
- Lyra provides hMPV in combination with RSV
- Cost?

# Thanks to:

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- John Ng
- Lily Law
- Linda Wong



"I'm afraid I still have more questions than answers."

**Thank you for your attention!**