COMPARISON OF FOCUS DIAGNOSTICS AND IN HOUSE RT-RT-PCR FOR INFLUENZA DETECTION

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AIM / BACKGROUND

A comparison of Focus Diagnostics and in-house assays for detection of influenza A (including pandemic strain), influenza B and RSV was conducted.

Simplexa™ Flu A/B & RSV assay (MOL2600) from Focus Diagnostics is a multiplex real-time RT-PCR amplification system for simultaneous detection and discrimination of influenza A, influenza B and RSV on 3M™ Integrated Cycler (MOL1001 and MOL1011). The assay uses a pair of a bi-functional fluorescent probe-primer and a reverse primer for each analyte and the internal RNA control (4, 7).

3M™ Integrated Cycler is a rapid real-time PCR thermocycler for simultaneous processing of up to 96 samples. It uses a specially designed spin disc, as part of a Microfluidic Molecular System, for fluorometric simultaneous multi-analyte identification of nucleic acid targets in samples (4, 6).

METHODS

- A total of 154 nasopharyngeal swabs from 2006 to 2010, positive for influenza A, influenza B, RSV, adenovirus, enterovirus or negative were selected (Tables 1, 2, 3). All samples were previously stored at -70°C.
- Nucleic acids from all samples were extracted with QIAamp® MinElute® Virus Spin Kit (QIAGEN).

- Samples were tested in parallel:
  - for detection of influenza A and influenza B all 154 samples were tested:
  with Simplexa™ Flu A/B & RSV on 3M™ Integrated Cycler and with in-house multiplex (influenza A and B) real-time RT-PCR (1,3,5) on RotorGene6000 (QIAGEN)
  - for detection of RSV all 154 samples were tested:
  with Simplexa™ Flu A/B & RSV on 3M™ Integrated Cycler and with in-house conventional RT-PCR (2) on PE2700 (PerkinElmer).
  Same conserved gene targets were used for virus identification in parallel assays:
  matrix gene for influenza A and influenza B and M gene for RSV (1,2,3,4,5).

- Sensitivity of detection of influenza A and influenza B was compared by parallel testing of a series of viral RNA dilutions.
- Sensitivity of detection of RSV was not compared.

- Running time of parallel RT-PCR methods was compared.

RESULTS

- Method comparison agreement for influenza A (subtypes: seasonal H1, seasonal H3, pandemic H1 and total) is given in Table 1.
- Average Ct difference for sample, showing correlation between parallel methods for influenza A (subtypes: seasonal H1, seasonal H3, pandemic H1), is given in Graphs 1, 2, 3.

- Simplexa™ Flu A/B & RSV assay from Focus Diagnostics also detected avian influenza A, subtypes H5, H7 and H9 (Table 1).

- Method comparison agreement for influenza B (Victoria lineage, Yamagata lineage and total) is given in Table 2.
- Average Ct difference for sample, showing correlation between parallel methods for influenza B (total), is given in Graph 4.

- Method comparison agreement for RSV (subtypes A, B and total) is given in Table 3.
- Average Ct difference for sample, showing correlation between parallel methods for RSV, was not compared, because real-time RT-PCR and conventional RT-PCR methods were used as parallel methods and can not be compared in this manner.

- Specificity was demonstrated also with no influenza A, influenza B or RSV detection in previously characterized negatives (3/3) or positives for adenovirus (2/2) or enterovirus (2/2). All this samples tested negative in parallel methods.

- End point dilutions from parallel testing showed no significant difference in sensitivity of influenza A and influenza B detection between methods (Table 4). Limit of detection was reached at the same end point dilution in 6 runs out of 8 runs.

- Running time of Focus Diagnostics system is significantly shorter than running time of currently used in-house methods (Table 5).

Table 4: Results of parallel testing of viral RNA dilutions for comparison of sensitivity of influenza A and influenza B detection.

DETECTION OF	SAMPLE	METHOD	SAMPLE DILUTION						
			1	1:1	1*10-1	1*10-2	1*10-3	1*10-4	1*10-5
INF A	1	Simplexa[1]	POS	POS	POS	POS	POS	POS	NEG
		In-house[2]	POS	POS	POS	POS	POS	NEG	NEG
	2	Simplexa	POS	POS	POS	POS	POS	POS	NEG
		In-house	POS	POS	POS	POS	POS	POS	NEG
	3	Simplexa	POS	POS	POS	POS	POS	NEG	NEG
		In-house	POS	POS	POS	POS	POS	NEG	NEG
	4	Simplexa	POS	POS	POS	POS	POS	POS	NEG
		In-house	POS	POS	POS	POS	POS	POS	NEG
	5	Simplexa	POS	POS	POS	POS	POS	POS	NEG
		In-house	POS	POS	POS	POS	POS	POS	NEG
	6	Simplexa	POS	POS	POS	POS	POS	NEG	NEG
		In-house	POS	POS	POS	POS	POS	POS	NEG
INF B	7	Simplexa	POS	POS	POS	POS	POS	POS	NEG
		In-house	POS	POS	POS	POS	POS	POS	NEG
	8	Simplexa	POS	POS	POS	POS	NEG	NEG	NEG
		In-house	POS	POS	POS	POS	NEG	NEG	NEG

[1]Simplexa™ Flu A/B & RSV. [2]In-house multiplex real-time RT-PCR (1,3,5).

Table 5: Running time of parallel RT-PCR methods (nucleic acid extraction not included).

RT-PCR METHOD	RT-PCR (hours)	POST AMPLIFICATION PRODUCT DETECTION (hours)	TOTAL TIME (hours)
Simplexa™ Flu A/B & RSV	1	0	1
In-house metod (detection of influenza A and influenza B)[1]	2	0	2
In-house metod (detection of RSV)[2]	3	1,5[3]	4,5

[1]In-house multiplex real-time RT-PCR (1, 3, 5). [2]In-house conventional RT-PCR (2). [3]Gel-electrophoresis.

Table 1: Sample selection for parallel testing and results of parallel testing: **INFLUENZA A.**

TESTED SAMPLES[1,2]	NUMBER OF SAMPLES TESTED WITH PARALLEL METHODS[3]	NUMBER OF SAMPLES CONCORDANT IN PARALLEL METHODS[3]	NUMBER OF SAMPLES NOT CONCORDANT IN PARALLEL METHODS[3]	COMMENTS	METHOD COMPARISON AGREEMENT (%)
INF A / H1 seasonal[1]	21	21	0	All concordant in parallel methods.	100
INF A / H3 seasonal[1]	21	21	0	All concordant in parallel methods.	100
INF A / H1 pandemic[1]	69	68	1	- NEGATIVE with Simplexa™ Flu A/B & RSV - POSITIVE with in-house method, Ct = 37, 35[4]	98,5
INF A / H5[2]	1	1	0	All concordant in parallel methods.	100
INF A / H7[2]	1	1	0	All concordant in parallel methods.	100
INF A / H9[2]	1	1	0	All concordant in parallel methods.	100
INF A TOTAL	114	113	1		99

[1]Samples are nasopharyngeal swabs typed and subtyped at National Institute of Public Health, Laboratory for virology, Ljubljana, Slovenia on time of sample collection. Results were confirmed at WHO CC, Mill Hill, London UK.
[2]Samples are commercial egg isolates of avian influenza A, subtypes H5, H7, H9.
[3]Given result for detection and identification of influenza A in samples with parallel methods was INFLUENZA A: POSITIVE/NEGATIVE only (target in both assays is matrix gene).
[4]Interpretation of results for in-house method is as follows: Ct ≤ 38.00 is POSITIVE, 38.00<Ct<40.00 is EQU (repeat testing), Ct ≥ 40.00 is NEGATIVE.

Table 2: Sample selection for parallel testing and results of parallel testing: **INFLUENZA B.**

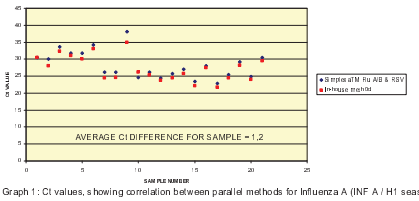
TESTED SAMPLES[1]	NUMBER OF SAMPLES TESTED WITH PARALLEL METHODS[2]	NUMBER OF SAMPLES CONCORDANT IN PARALLEL METHODS[2]	NUMBER OF SAMPLES NOT CONCORDANT IN PARALLEL METHODS[2]	COMMENTS	METHOD COMPARISON AGREEMENT (%)
INF B, Victoria lin.[1]	16	16	0	All concordant in parallel methods.	100
INF B, Yamagata lin.[1]	1	1	0	All concordant in parallel methods.	100
INF B TOTAL	17	17	0		100

[1]Samples are nasopharyngeal swabs typed and subtyped at National Institute of Public Health, Laboratory for virology, Ljubljana, Slovenia on time of sample collection. Results were confirmed at WHO CC, Mill Hill, London UK.
[2]Given result for detection and identification of influenza B in samples with parallel methods was INFLUENZA B: POSITIVE/NEGATIVE only (target in both assays is matrix gene).

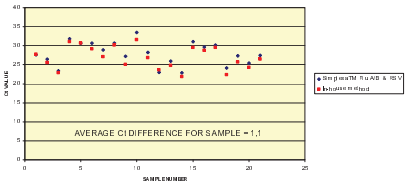
Table 3: Sample selection for parallel testing and results of parallel testing: **RSV.**

TESTED SAMPLES[1]	NUMBER OF SAMPLES TESTED WITH PARALLEL METHODS[2]	NUMBER OF SAMPLES CONCORDANT IN PARALLEL METHODS[2]	NUMBER OF SAMPLES NOT CONCORDANT IN PARALLEL METHODS[2]	COMMENTS	METHOD COMPARISON AGREEMENT (%)
RSV A[1]	5	5	0	All concordant in parallel methods.	100
RSV B[1]	2	2	0	All concordant in parallel methods.	100
RSV A and B[1]	2	2	0	All concordant in parallel methods.	100
RSV not subtyped[1]	7	7	0	All concordant in parallel methods.	100
RSV TOTAL	16	16	0		100

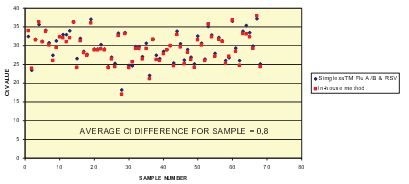
[1]Samples are nasopharyngeal swabs typed and subtyped at National Institute of Public Health, Laboratory for virology, Ljubljana, Slovenia on time of sample collection and samples from an external quality assessment panel.
[2]Given result for detection and identification of RSV in both parallel methods was RSV: POSITIVE/NEGATIVE only (target in both assays is M gene).



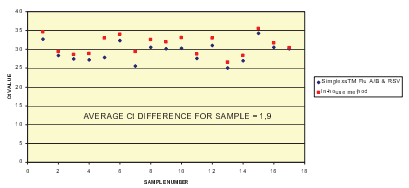
Graph 1: Ct values, showing correlation between parallel methods for Influenza A (INF A / H1 seasonal).



Graph 2: Ct values, showing correlation between parallel methods for Influenza A (INF A / H3 seasonal).



Graph 3: Ct values, showing correlation between parallel methods for Influenza A (INF A / H1 pandemic).



Graph 4: Ct values, showing correlation between parallel methods for Influenza B.

CONCLUSIONS

Focus Diagnostics system composed of Simplexa™ Flu A/B & RSV assay on 3M™ Integrated Cycler showed:
- overall good correlation with currently used in-house methods
- overall good specificity and sensitivity in comparison to currently used in-house methods
- results are available in a significantly shorter time in comparison to currently used in-house methods
- results for all analytes are available simultaneously in comparison to currently used in house methods.

Simplexa™ Flu A/B & RSV assay on 3M™ Integrated Cycler is a reliable system for rapid differential diagnostics of influenza A (including pandemic strain), influenza B and RSV.

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