

Surveillance of Respiratory Syncytial Virus Global Influenza Surveillance and Response System (GISRS) Platform

External Quality Laboratory Assessment

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**WHO MEETING Mid-term Review of the RSV Surveillance Pilot
Global Influenza Surveillance and Response System (GISRS)**

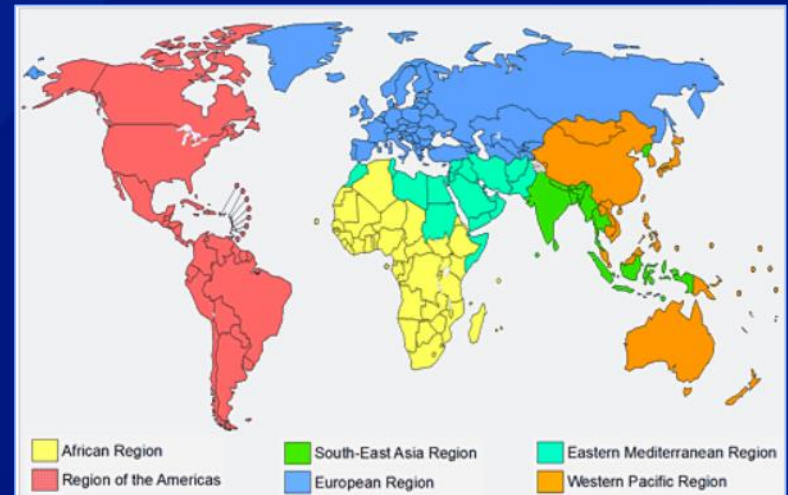
Washington, DC December 19 2017

**The findings and conclusions of this presentation are those of the author(s) and do not necessarily
represent the official position of the Centers for Disease Control and Prevention**

December 2016

Country Selection

Country	Region	Country
1	AFRO	Côte d'Ivoire
2		Mozambique
3		South Africa
4	EMRO	Egypt
5		Oman
6	EURO	Russia
7	PAHO	Argentina
8		Brazil
9		Chile
10		Canada
11	SEARO	India
12		Thailand
13	WPRO	Mongolia



Laboratory Implementation WHO RSV Initiative

- Pre-survey questionnaire
- Material transfer agreement (MTA)
- Protocols
 - ✓ troubleshooting
- Qualified reagents
- Commercial invoices
- Import permits
- EQA/ Proficiency panels (+/- and detection range)
 - ✓ CDC real-time RT-PCR assay
 - ✓ Laboratory Developed Test (LDT)
 - ✓ Commercial assays
- Distribution of real-time RT-PCR reagents and EQA proficiency panels
- Reporting EQA/ Proficiency panels (CDC RSV EQA Report Form)
- Troubleshooting



RSV Real-time RT-PCR Assay

CDC EQA Proficiency Testing Programs

CDC Respiratory syncytial virus (RSV) and Human RNase P control (RNP) Real-time RT-PCR Primers/Probes

CATALOG: KK0813

EXPIRATION DATE: 09/01/2018

INTENDED USE: CDC real-time RT-PCR primer/probes are intended for the *in vitro* qualitative detection of respiratory syncytial virus (RSV) and Human RNase P control (RNP).

USE LIMITATIONS: For research use only. Not intended for clinical diagnostic use.

REAGENTS:

****DO NOT DISCARD: Important product-specific information****

CDC Respiratory Syncytial Virus (RSV) RT-PCR Positive Control

LOT: TX050616

INTENDED USE: For use as positive control for CDC real-time RT-PCR assays for detection of respiratory syncytial virus and human ribonuclease P (RNase P).

USE LIMITATIONS: For research use only. Not intended for clinical diagnostic use.

****DO NOT DISCARD: Important Product-Specific Information****

CDC Respiratory Syncytial Virus (RSV) Real-time RT-PCR Assay 2016 External Quality Assessment Panel

Notice: Do not use until notified by CDC. Store under conditions indicated below.

LOT: RSV-PT001, lot # 52010

EXPIRATION DATE: 09/1/2018

INTENDED USE: The CDC External Quality Assessment (EQA) Panel is for evaluation of the performance of Real-time RT-PCR Assays for Respiratory Syncytial Virus (RSV).

USE LIMITATIONS: For research use only. Not intended for diagnostic purposes. Do not reuse. Do not redistribute.

REAGENT: Each EQA Panel consists of 20 (twenty) lyophilized mock human respiratory specimens spiked with inactivated cell cultured RSV.

Primers and probes:

CDC Respiratory syncytial virus (RSV) (2)

CDC Human RNase P control (RNP) (2)

Synthetic positive control (1)

Real-time RT-Assay External Quality Assessment Panel

Lyophilization /freeze-drying

Reduced shipping cost,
any suitable courier

20 samples

“proxy” assay testing

2 historical RSV strains (A and B)

2 recent RSV strains (A and B)

Results:

Positive/negative, reproducibility,
RSV assays

(CDC x LDT , commercial assays)



CDC RSV External Quality Assessment Panel (EQA)

RSV Group	Strain Name	Genotype	Sample No.	Duplicate	Expected CT Values		Results
					RSV	RNP	
	A	Strain Long	1		≤ 32	≤ 33	RSV Pos
	A	Strain Long	2	2 = 8	≤ 30	≤ 34	RSV Pos
	A	Strain Long	3		≤ 26	≤ 34	RSV Pos
		Blank	4		Neg	≤ 33	RSV Neg
	B	Strain 209_GB3	5		≤ 28	≤ 31	RSV Pos
	B	Strain 209_GB3	6		≤ 32	≤ 29	RSV Pos
	B	Strain 209_GB3	7	7 = 13	≤ 35	≤ 33	RSV Pos
	A	Strain Long	8	8 = 2	≤ 30	≤ 34	RSV Pos
		Blank	9		Neg	≤ 33	RSV Neg
	A	Strain 15353_ON1	10		≤ 37	≤ 33	RSV Pos
	A	Strain 15353_ON1	11	11 = 18	≤ 29	≤ 33	RSV Pos
	A	Strain 15353_ON1	12		≤ 27	≤ 33	RSV Pos
	B	Strain 209_GB3	13	13 = 7	≤ 35	≤ 33	RSV Pos
		Blank	14		Neg	≤ 33	RSV Neg
	B	Strain 53530_BA	15	15 = 20	≤ 26	≤ 31	RSV Pos
	B	Strain 53530_BA	16		≤ 28	≤ 31	RSV Pos
	B	Strain 53530_BA	17		≤ 34	≤ 32	RSV Pos
	A	Strain 15353_ON1	18	18 = 11	≤ 29	≤ 33	RSV Pos
		Blank	19		Neg	≤ 34	RSV Neg
	B	Strain 53530_BA	20	20 = 15	≤ 26	≤ 31	RSV Pos

RSV External Quality Assessment (1)

Summary

- 20 samples Positive (16) / Negative (4), Duplicates (4)
- Positive & Negative samples:
 - ✓ 12/13 CDC assay
 - ✓ 1/13 false positive (“pipetting error” not contamination during NA extraction)
- Duplicates:
 - ✓ 12/13 (2-8), 8/13 (7-13), 11/13 (11-18), 12/13 (15-20)
- CDC x LDT / Commercial assays :
 - ✓ CDC x LDT: 2 laboratories (pan RSV and a RSV type/subgroup assay)
 - ✓ CDC x commercial assays:
 - 4 laboratories: assays “A” (2), “B” (1), “C” (1)
- Recommendation (Jan-March 2017):
 - ✓ Troubleshooting Ct values (4/13) > Request Run files > Analysis
 - ✓ CDC & LDT : replace LDT with CDC assay (1 laboratory)
 - ✓ CDC & Commercial assay:
 - replace commercial assays “B” and “C” with CDC assay (2 laboratories)

RSV External Quality Assessment (2)

Laboratory Developed Test (LDT)

rRT PCR RSV External Quality Assessment / Proficiency Panel

RSV Group	Strain Name	Genotype	Sample No.	Duplicate	Expected CT Values		Results
					RSV	RNP	
A	Strain Long		1		≤ 32	≤ 33	RSV Pos
A	Strain Long		2	2 = 8	≤ 30	≤ 34	RSV Pos
A	Strain Long		3		≤ 26	≤ 34	RSV Pos
	Blank		4		Neg	≤ 33	RSV Neg
B	Strain 209_GB3		5		≤ 28	≤ 31	RSV Pos
B	Strain 209_GB3		6		≤ 32	≤ 29	RSV Pos
B	Strain 209_GB3		7	7 = 13	≤ 35	≤ 33	RSV Pos
A	Strain Long		8	8 = 2	≤ 30	≤ 34	RSV Pos
	Blank		9		Neg	≤ 33	RSV Neg
A	Strain 15353_ON1		10		≤ 37	≤ 33	RSV Pos
A	Strain 15353_ON1		11	11 = 18	≤ 29	≤ 33	RSV Pos
A	Strain 15353_ON1		12		≤ 27	≤ 33	RSV Pos
B	Strain 209_GB3		13	13 = 7	≤ 35	≤ 33	RSV Pos
	Blank		14		Neg	≤ 33	RSV Neg
B	Strain 53530_BA		15	15 = 20	≤ 26	≤ 31	RSV Pos
B	Strain 53530_BA		16		≤ 28	≤ 31	RSV Pos
B	Strain 53530_BA		17		≤ 34	≤ 32	RSV Pos
A	Strain 15353_ON1		18	18 = 11	≤ 29	≤ 33	RSV Pos
	Blank		19		Neg	≤ 34	RSV Neg
B	Strain 53530_BA		20	20 = 15	≤ 26	≤ 31	RSV Pos

rRT PCR RSV External Quality Assessment / Proficiency Panel Results

Sample No.	CDC Real-time RT-PCR assay (Ct values)		Real-time RT-PCR assay currently in use by the lab (Ct values)		Results	Expected Values (Cts)			Results
	RSV	RNP	RSV †	RSV A / RSV B ‡		RNP †	RSV	RNP	
1	31	35		32 (A)	34	Positive	≤ 32	≤ 33	Pos RSV
2	30	37		31 (A)	35	Positive	≤ 30	≤ 34	Pos RSV
3	26	36		27 (A)	37	Positive	≤ 26	≤ 34	Pos RSV
4	Neg	35		Neg	36	Negative	Neg	≤ 33	Neg RSV
5	30	34		32 (B)	33	Positive	≤ 28	≤ 31	Pos RSV
6	32	34		34 (B)	33	Positive	≤ 32	≤ 29	Pos RSV
7	35	35		38 (B)	33	Positive	≤ 35	≤ 33	Pos RSV
8	32	35		32 (A)	34	Positive	≤ 30	≤ 34	Pos RSV
9	38	36		Neg	36	Inconclu	Neg	≤ 33	Neg RSV
10	38	34		38 (A)	34	Positive	≤ 37	≤ 33	Pos RSV
11	30	34	A	30 (A)	34	Positive	≤ 29	≤ 33	Pos RSV
12	26	34		26(A)	34	Positive	≤ 27	≤ 33	Pos RSV
13	28	36		29(B)	34	Positive	≤ 35	≤ 33	Pos RSV
14	Neg	35		38(A)	36	Inconclu	Neg	≤ 33	Neg RSV
15	26	34		29(B)	35	Positive	≤ 26	≤ 31	Pos RSV
16	30	36	B	33(B)	37	Positive	≤ 28	≤ 31	Pos RSV
17	34	37		37(B)	35	Positive	≤ 34	≤ 32	Pos RSV
18	30	34		30(A)	34	Positive	≤ 29	≤ 33	Pos RSV
19	Neg	36		Neg	36	Negative	Neg	≤ 34	Neg RSV
20	27	35	B	29(B)	39	Positive	≤ 26	≤ 31	Pos RSV
PC	30	32		26(A)23(B)	27	Positive	≤ 35	< 34	Positive
NC	Neg	Neg		Neg	Neg	Neg	Neg	≤ 34	Neg RSV

‡ Duplex real-time RT-PCR: RSV and RNP

† Duplex real-time RT-PCR: RSV A and B

References:

Gunson et al. 2005. J.Clin. Virol. 33, 341-344.

Alma C. van de Pol et al. 2010. J.Clin. Microbiol. 48, 3569-3574.

R. Brittain-Long et al. 2008. J. Clin. Virol. 41, 53-56.

RSV External Quality Assessment (4)

RSV Commercial Assay "C"

rRT PCR RSV External Quality Assessment / Proficiency Panel

RSV Group	Strain Name	Genotype	Sample No.	Duplicate	Expected CT Values		Results
					RSV	RNP	
A	Strain Long		1		≤ 32	≤ 33	RSV Pos
A	Strain Long		2	2 = 8	≤ 30	≤ 34	RSV Pos
A	Strain Long		3		≤ 26	≤ 34	RSV Pos
	Blank		4		Neg	≤ 33	RSV Neg
B	Strain 209_GB3		5		≤ 28	≤ 31	RSV Pos
B	Strain 209_GB3		6		≤ 32	≤ 29	RSV Pos
B	Strain 209_GB3		7	7 = 13	≤ 35	≤ 33	RSV Pos
A	Strain Long		8	8 = 2	≤ 30	≤ 34	RSV Pos
	Blank		9		Neg	≤ 33	RSV Neg
A	Strain 15353_ON1		10		≤ 37	≤ 33	RSV Pos
A	Strain 15353_ON1		11	11 = 18	≤ 29	≤ 33	RSV Pos
A	Strain 15353_ON1		12		≤ 27	≤ 33	RSV Pos
B	Strain 209_GB3		13	13 = 7	≤ 35	≤ 33	RSV Pos
	Blank		14		Neg	≤ 33	RSV Neg
B	Strain 53530_BA		15	15 = 20	≤ 26	≤ 31	RSV Pos
B	Strain 53530_BA		16		≤ 28	≤ 31	RSV Pos
B	Strain 53530_BA		17		≤ 34	≤ 32	RSV Pos
A	Strain 15353_ON1		18	18 = 11	≤ 29	≤ 33	RSV Pos
	Blank		19		Neg	≤ 34	RSV Neg
B	Strain 53530_BA		20	20 = 15	≤ 26	≤ 31	RSV Pos

rRT PCR RSV External Quality Assessment / Proficiency Panel Results

Sample No.	CDC Real-time RT-PCR assay (Ct values)		Real-time RT-PCR assay currently in use by the lab (Ct values) ***		Results	Expected Values (Cts)		
	RSV	RNP	RSV	RNP		RSV	RNP	Results
1	33.4	30.1	29 (RSVA)	33.2	Positive	≤ 32	≤ 33	Pos RSV
2	29.2	30.4	30.1 (RSVA)	31.4	Positive	≤ 30	≤ 34	Pos RSV
3	23.9	30	24 (RSVA)	33.3	Positive	≤ 26	≤ 34	Pos RSV
4	Neg	30.2	Neg	30.3	Negative	Neg	≤ 33	Neg RSV
5	28.6	30	28.8 (RSVB)	30.6	Positive	≤ 28	≤ 31	Pos RSV
6	33.4	30.6	34.1 (RSVB)	30.3	Positive	≤ 32	≤ 29	Pos RSV
7	34.3	31.4	35.3 (RSVB)	30.2	Positive	≤ 35	≤ 33	Pos RSV
8	30.7	32.3	29.4 (RSVA)	32.4	Positive	≤ 30	≤ 34	Pos RSV
9	Neg	29.6	Neg	31.1	Negative	Neg	≤ 33	Neg RSV
10	34.8	30.3	41.4 (RSVA)	31	Positive	≤ 37	≤ 33	Pos RSV
11	27.5	29.2	30.9 (RSVA)	31.7	Positive	≤ 29	≤ 33	Pos RSV
12	23.5	29.8	27.2 (RSVA)	34.4	Positive	≤ 27	≤ 33	Pos RSV
13	30.5	31	33.9 (RSVB)	30.9	Positive	≤ 35	≤ 33	Pos RSV
14	Neg	30.1	Neg	31.4	Negative	Neg	≤ 33	Neg RSV
15	24.7	29.3	29.8 (RSVB)	35.6	Positive	≤ 26	≤ 31	Pos RSV
16	28.2	29.9	33 (RSVB)	35.6	Positive	≤ 28	≤ 31	Pos RSV
17	30.6	28.8	33.3 (RSVB)	31.4	Positive	≤ 34	≤ 32	Pos RSV
18	25.7	29	28.6 (RSVB)	34.2	Positive	≤ 29	≤ 33	Pos RSV
19	Neg	29.4	Neg	31.1	Negative	Neg	≤ 34	Neg RSV
20	24	28.6	27.4 (RSVB)	32.8	Positive	≤ 26	≤ 31	Pos RSV
PC	26.1	25.1	27 (RSVA) & 21 (RSVB)	22	Positive	≤ 35	< 34	Positive
NC	Neg	Neg	Neg	Neg	Negative	Neg	≤ 34	Neg RSV

Acknowledgments:

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