

Results of the MolecularDiagnostics.be study group: storage conditions of respiratory samples

Anne Vankeerberghen¹, Kurt Beuselincx², Patrick Descheemaeker³, Marie-Jeanne Stouten⁴, Dominique Ursi⁵, Ben Vanmassenhove⁶, Hans De Beenhouwer¹
¹OLVZ Aalst, Aalst, Belgium, ²UZ Leuven, Leuven, Belgium, ³AZ Sint-Jan Brugge-Oostende AV, Brugge, Belgium, ⁴Hopital Saint-Pierre, Brussel, Belgium, ⁵UZA, Antwerpen, Belgium, ⁶AZ Damiaan, Oostende, Belgium.

Introduction and purpose

MolecularDiagnostics.be is an organization of Molecular Biologists in Belgium affiliated to Hospital laboratories and active in the field of Molecular Diagnostics. Scientific meetings, ring controls and discussion forums are organised aiming to improve the quality of molecular diagnostics.

ISO15189 demands validation and description of storage conditions for samples, during transport, before analysis and after analysis in the lab, that guarantee stability of the relevant target. Since there is no clear guideline for the storage of respiratory samples for molecular diagnostics, a Belgian national multicentre study was conducted by MolecularDiagnostics.be between January and May 2014.

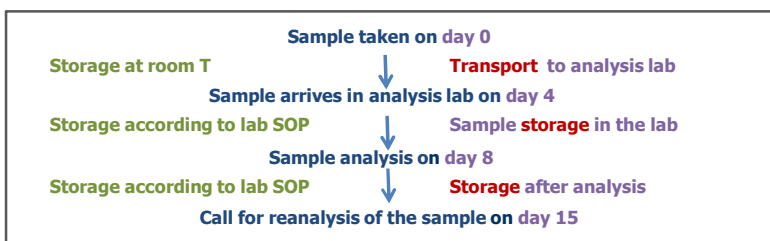
Methods

Study protocol

A protocol was set up

- to mimick transport at room temperature
- to validate storage conditions in use in the lab (before and after analysis)
- using the real time PCR method in use in routine setting in the participating lab
- for all respiratory pathogens tested in the lab

MolecularDiagnostics.be members were asked to store the samples according to the following scheme and to analyse an aliquot of the sample at the different time points. Six laboratories participated. Ct values were analysed by the organising lab.



Sample types analyzed:

BAL, bronchus / tracheal aspirate, sputa, nasopharyngeal swab (in UTM), nasopharyngeal aspirate, throat swab

Conclusion

Although respiratory samples = heterogenous samples, few variations in Ct were found. This indicates that bacterial and viral DNA, and viral RNA are stable in the different sample types analysed, with the different analysis methods used, and under the different storage conditions

Storage / analysis conditions used:

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6
at arrival:	2-8°C	2-8°C	RT	2-8°C	RT	2-8°C
day 0 - day 4	RT	RT	RT	RT	RT	RT
day 4 - day 8	2-8°C	2-8°C	3-8°C	not done	2-8°C	2-8°C
day 8 - day 15	2-8°C	2-8°C	-20°C	-80°C	-80°C	2-8°C
number of samples:	16	1	24	3	35	2
extraction method:	EasyMAG	EasyMAG	QIASym-phony	QIASym-phony	EasyMAG	MagNA Pure
PCR method	In house RT PCR	In house PCR	Respicard	Argene	In house RT PCR	In house RT PCR

Results

1. Stability of bacterial DNA: *M. pneumoniae*

Sample type:	sputum	BAL	swab URT	swab URT	swab URT
Analysis at arrival	22,56	20,6	36,4	30,1	31,9
Analysis at day 4 (RT)	20,12	20,4	35,1	26	31,5
Analysis at day 8 (2-8°C)	20,11	19,6	36	/	32,7
Analysis at day 15 (2-8°C)	19,97				
Analysis at day 15 (-80°C)		19,5	/	/	31,7
dCt (last-day 0)	-2,59	-1,1			-0,2

2. Stability of bacterial DNA: *Bordetella* DNA

Sample type:	NPA swab (pertussis)	Swab URT (parapertussis)
Analysis at arrival	20,51	23,55
Analysis at day 4 (RT)	20,81	23,67
Analysis at day 8 (2-8°C)	20,68	22,49
Analysis at day 15 (2-8°C)	20,16	/
dCt (last-day 0)	-0,35	

3. Stability of viral DNA: Adenovirus

Sample type:	NPA	UTM	BA	UTM	BAL	UTM	NPA	NPA	UTM	UTM
Analysis at arrival	14,33	27,4	36,1	17	31,4	15	19,3	16,2	23	18,4
Day 4 (RT)	14,92	28,3	35,3	16,7	34,6	15,2	19,6	16,3	24	20,7
Day 8 (2-8°C)	11,61	29,4	/	17,3	/	15,3	19,5	15,4	/	17,7
Day 15 (2-8°C)	10,83									
Day 15 (-20°C)		28,6	/	16,7	/	/	/	14,7	25,27	20,9
dCt (last-day 0)	-3,5	1,2		-0,3				-1,5	2,27	2,5

4. Stability of viral DNA: Bocavirus

Sample type:	NPA	NPA
Analysis at arrival	13,71	31,21
Analysis at day 4 (RT)	11,78	30,18
Analysis at day 8 (2-8°C)	10,46	30,22
Analysis at day 15 (2-8°C)	11,08	30,87
dCt (last-day 0)	-2,63	-0,34

5. Stability of viral RNA: Influenza A virus

Sample type:	NPA swab	NPA swab	NPA	Sputum	UTM	UTM	UTM
Analysis arrival	25,16	23,09	17,51	15,93	35,4	27,8	25,4
Analysis day 4 (RT)	27,18	22,41	19,8	17,67	35,3	28,2	27,2
Analysis day 8 (2-8°C)	29,82	23,66	18,75	17,55	35,7	25,2	30,3
Analysis day 15 (2-8°C)	30,99	22,94	18,5	18,27			
Analysis day 15 (-20°C)					36,3	31,2	28,2
dCt (last-day 0)	5,83	-0,15	0,99	2,34	0,9	3,4	2,8

Influenza A storage at day 15 (-80°C):

Mean dCt (n=20) :	0,27 Ct
Range:	-1,5 - 1,8
Samples:	19 NPA swabs + 1 BA

6. Stability of viral RNA: hMPV

Sample type:	Sputum	NPA	NPA	NPA	NPA	NPA	NPA	NPA	NPA	NPA
Analysis arrival	22,27	32,13	22,89	27,79	33,88	27,02	18,7	20,7	28,3	35,3
Analysis day 4 (RT)	23,07	31,66	26,15	31,02	34,01	24,29	18,1	22,8	29,1	37,2
Analysis day 8 (2-8°C)	22,71	31,13	24,14	27,8	/	/	/	/	27,4	34,3
Analysis day 15 (2-8°C) /	/	/	24,53	/						
Analysis day 15 (-80°C)					32,74	28,48	18,9	/	/	34
dCt (last-day 0)			1,64		-1,14	1,46	0,2			-1,3

7. Stability of viral RNA: RSV

Sample type:	NPA	NPA	BAL	BAL	BAL	BA	NP swab
Analysis at arrival	32,62	22,92	29,7	34,7	16,1	25	34,7
Analysis at day 4 (RT)	33,78	28,87	27,4	35,3	16,1	26	neg
Analysis at day 8 (2-8°C)	34,25	23,89	27	/	16,1	25,3	neg
Analysis at day 15 (2-8°C)	33,25	23	27,2				
Analysis at day 15 (-80°C)						26,5	neg
dCt (last-day 0)	0,63	0,08	-2,5	0,6	0	1,5	