

Analytical performance of the PreTect HPV-Proofer assay for detection of E6/E7 mRNA expression and typing of 5 high-risk HPVs

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Objective

To investigate the analytical performance of the PreTect HPV-Proofer assay for detection of E6/E7 mRNA expression from the high-risk HPV types 16, 18, 31, 33 and 45. We evaluated the following parameters: variability, reproducibility, analytical sensitivity, analytical intra- and inter-species specificity, stability of RNA after storage and performance of artificial positive controls.

Methods

Cervical cell samples were collected from a brush after performance of cervical smears. Samples containing HPV from the relevant HPV types were selected for further analysis. HPV 16 and 18 RNA was also obtained from SiHa, CaSki and HeLa cell lines and by *in vitro* transcription of pGEM-T Easy vectors containing HPV DNA constructs covering the open reading frame of E6/E7 (BaseClear B.V. Leiden, The Netherlands). The extracted RNA was stored at -70°C until RNA amplification using PreTect HPV-Proofer.

Results

Variability was found to be low between runs (Table 1), within run, between days and between readers from different manufacturers. Mean reproducibility was found to be 92-100% depending on HPV type (Table 2). The positive controls were stable in 30 parallel runs showing a 100% hit rate. mRNA was successfully amplified even when stored for more than one year. Analytical sensitivity for detection of mRNA was 100 SiHa or CaSki cells, 5 HeLa cells (Table 3) and 10 copies of HPV 16 mRNA and 100 copies of HPV 18 mRNA in cell free systems. No cross reactivity was seen with other HPV types (Table 4) or with 7 of the most common infections of the anogenital tract: *Gardnerella vaginalis*, *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Candida*, *Herpes simplex type II*, *Neisseria gonorrhoeae*, and *Mycoplasma hominis*.

Discussion

The variability of all parameters investigated is low and reproducibility is high indicating a good stability of the performance of PreTect HPV-Proofer. Analytical sensitivity was found to be very high and lack of cross reactions with other HPV types or other infections commonly found in the anogenital tract indicates a high analytical specificity. Indirect evidence for detection of mRNA and not DNA is given by the fact that PreTect HPV-Proofer detected equal numbers of SiHa and CaSki cells despite the considerable differences in number of DNA copies (SiHa: 1-2 DNA copies, CaSki: 600 DNA copies).

Table 1: Variability between runs

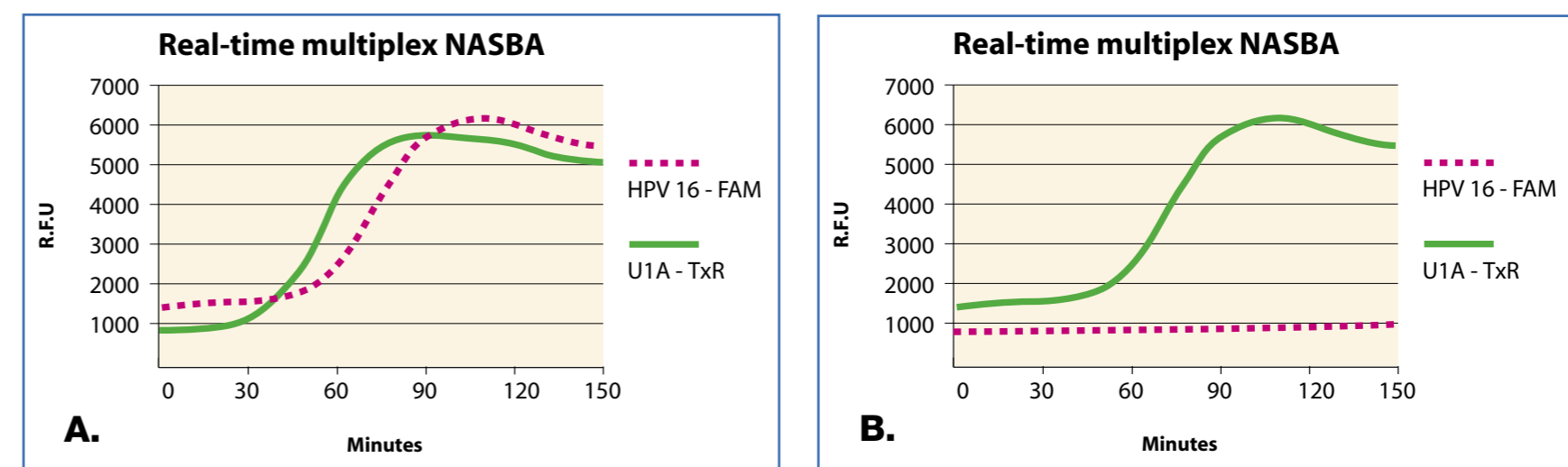
HPV type	Sample no	Run 1	Run 2	Run 3	Mean run 1, 2, 3
16	1*	1,59	2,04	0,92	1,52
	2	3,32	3,4	3,83	3,52
	3	3,56	3,6	4,04	3,73
18	1	2,52	1,98	2,64	2,38
	2	2,64	2,74	2,65	2,68
	3*	2,13	0,68	2,38	1,73
31	1	2,34	2,87	2,16	2,46
	2	3,26	3,12	3,49	3,29
	3	3	2,68	3,21	2,96
33	4*	0,89	2,16	0,78	1,28
	1	4,09	4,36	4,24	4,23
	2	3,59	4,15	3,87	3,87
45	3	3,76	4,19	3,86	3,94
	4	3,17	4,01	3,78	3,65
	1	4,97	5,58	5,22	5,26
U1A	2	5,28	5,36	5,16	5,27
	3	5,24	5,49	5,63	5,45
	4	3,54	4,68	4,95	4,39
U1A	1	2,91	2,64	2,94	2,83
	2	2,81	2,83	2,95	2,86
	3	2,8	2,85	2,88	2,84

* Some of the samples seemed to be "unstable", probably due to a low amount of transcripts or viral load as increase in signal was consistently low.

Table 2. Reproducibility of PreTect HPV-Proofer

HPV Type	# Samples	Runs per sample	Mean reproducibility
HPV 16	7	13 - 26	92.3%
HPV 18	7	7 - 26	99.5%
HPV 31	4	16 - 26	95.3%
HPV 33	7	11 - 27	100.0%
HPV 45	7	9 - 27	100.0%

Figure 1. Real-time multiplex Nucleic Acid Sequence Based Amplification (NASBA)



A. Sample positive for HPV 16 mRNA and for the human U1A mRNA internal sample control.
 B. Sample negative for HPV 16 mRNA but positive for the human U1A mRNA internal sample control.

Table 3. Analytical sensitivity of PreTect HPV-Proofer in a background of HPV negative cells

# HPV positive cells	HPV 16		HPV 16		HPV 18	
	Cell line	Hit rate	Cell line	Hit rate	Cell line	Hit rate
5	CaSki	ND	SiHa	ND	HeLa	100%
10	CaSki	30%	SiHa	0%	HeLa	100%
100	CaSki	100%	SiHa	100%	HeLa	100%
1000	CaSki	100%	SiHa	100%	HeLa	100%

The analytical sensitivity was tested in a background of 10 000 HPV negative cells (breast cancer cell line MA-II). ND = Not Detected.

Table 4. Analytical specificity for PreTect HPV-Proofer

PreTect HPV-Proofer	PCR								Total
	HPV 6/11	HPV 16	HPV 18	HPV 31	HPV 33	HPV 35	HPV 45	HPV 51	
HPV 16	0	21	0	0	0	0	0	0	0
HPV 18	0	0	17	0	0	0	0	0	0
HPV 31	0	0	0	7	0	0	0	0	0
HPV 33	0	0	0	0	4	0	0	0	0
HPV 45	0	0	0	0	0	0	10	0	0
Total tested	25	21	17	7	4	7	10	10	101

Figure 3. Principle of NASBA

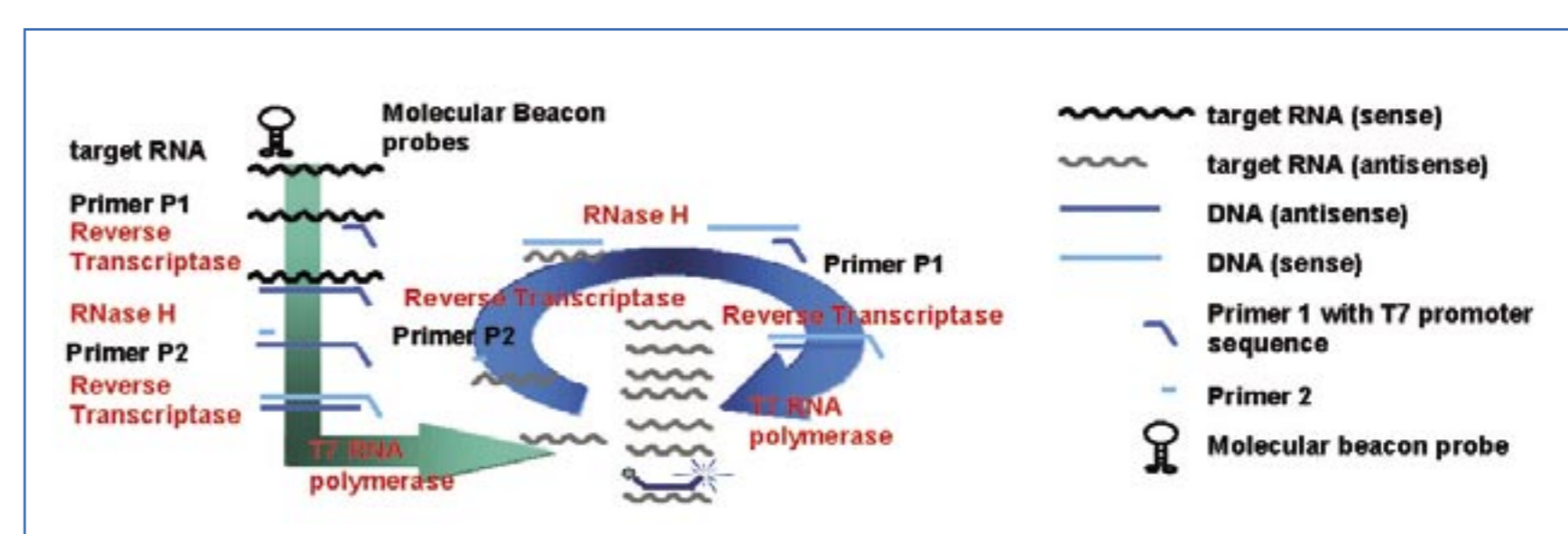
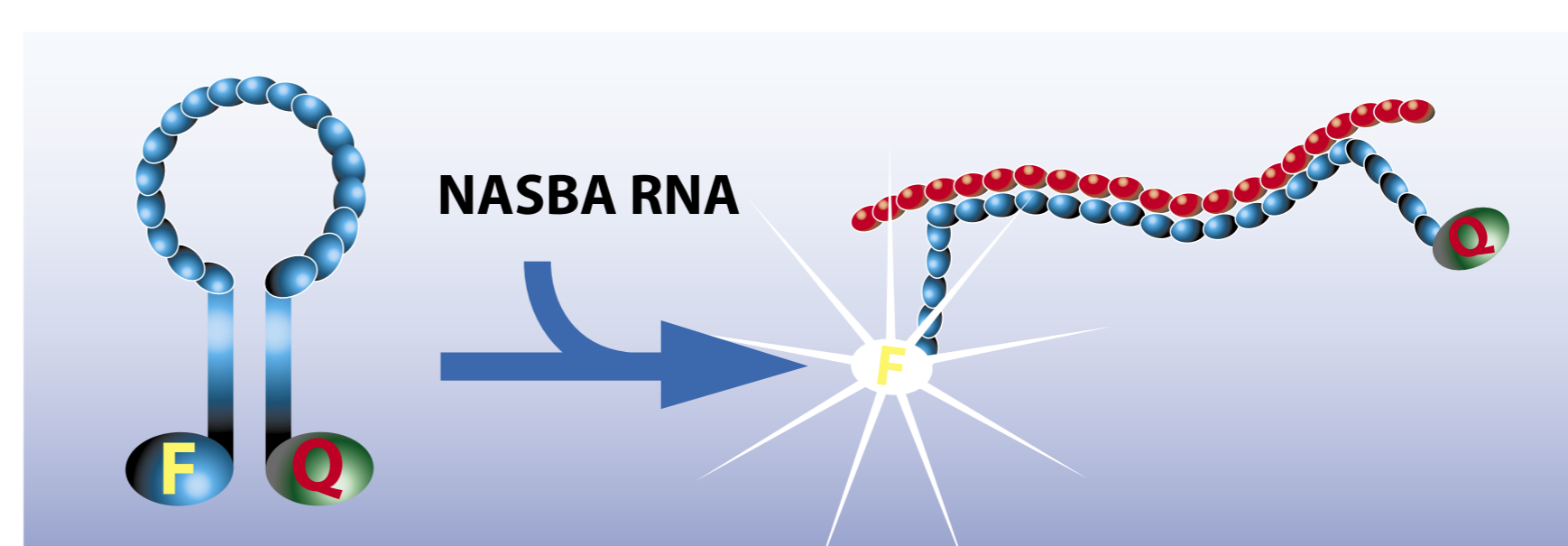


Figure 3. Principle of Molecular Beacon



Molecular Beacons are DNA probes with modified ends. In the folded state (stem-loop) the fluorophore is quenched, but upon binding of the loop sequence to its complementary target sequence the probe undergoes a conformational change and a fluorescence signal is emitted. The probes will hybridize to the anti-sense RNA transcripts that are produced during the transcriptional phase of the NASBA reaction. While amplification proceeds, fluorescent signals are measured real-time in a fluorescent reader.

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