

# High quality mRNA can be extracted from routinely collected cervical samples by several commercially available methods and detected by PreTect HPV-Proofer

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## Objective

Due to a supposedly higher instability of mRNA compared to DNA in clinical samples, it is of interest to verify the quality of mRNA in routinely collected cervical samples. The objective of this study was to investigate the quality of total RNA extracted with 9 routine based commercially available extraction methods.

## Methods

Cervical cell samples were collected from a Cervex-brush and stored at room temperature in PreServ Cyt (Cytoc Corp, MA, USA) until extraction of total RNA, but for no longer than two weeks. The extraction methods included are shown in table 1 and fig 1. Five of 20 ml cell suspension was used for extraction of DNA and/or RNA. Before nucleic acid isolation by the methods based on spin columns, the cells were washed once in 1ml of 96% ethanol.

Quality of mRNA was assessed by amplification of the human U1A gene product (U1 small nuclear ribonucleoprotein A, a housekeeping gene). Amplification was performed by the NASBA-based PreTect HPV-Proofer Assay utilising real-time detection by molecular beacons (Fig 2 and 3).

## Results

mRNA was successfully recovered in 98-100% of cases. Amplification results show high amounts of human mRNA in the cervical samples. Successful detection of U1A expression was verified for all extraction methods included in the study (Table 2). The extraction methods used needed only minor adjustments to compensate for e.g. buffer requirements of the PreTect HPV-Proofer assay. In U1A negative samples, the number of cells was found to be low (on average 6 cells/ $\mu$ l for U1A negative samples compared to 113 cells/ $\mu$ l for U1A positive samples).

## Discussion

Recovery of mRNA by all extraction methods, including automatic high throughput systems, indicates that RNA is well preserved and suited as a target for routine clinical analysis. In U1A positive samples, HPV RNA has also been stably detected (1). Therefore, expression from HPV E6/E7 genes can easily be monitored in cervical samples. However, preservation of the cells in the collection buffer is important and buffers containing methanol is well suited for this purpose. Commercially available extraction methods both give high quality RNA and an opportunity for standardization of the laboratory operating procedures.

Table 1: Extraction methods

Method	Manufacturer	Type	Based on	Nucleic acids isolated	Elution volume	Lysisbuffer (volume)
RNAquous-Micro	Ambion, Austin TX, USA	manual	Spin column	RNA	40 $\mu$ l	300 $\mu$ l
M48 Biorobot. Magattract RNA cell mini kit	Qiagen, Hilden Germany	automated	Magnetic silica	RNA/ DNA	50 $\mu$ l	400 $\mu$ l
RNeasy mini kit.	Qiagen, Hilden Germany	manual	Spin column silica based	RNA	40 $\mu$ l	350 $\mu$ l
Perfect RNA, Eucaryotic, Mini	Eppendorf AG, Hamburg Germany	manual	Spin column silica based	RNA	50 $\mu$ l	350 $\mu$ l
RNA-spin	Intron Biotechnology, Seoul, South Korea	manual	Spin column silica based	RNA	40 $\mu$ l	350 $\mu$ l
Versagene, RNA cell kit	Genra, Minneapolis, Minnesota, USA	manual	Spin column silica based	RNA	50 $\mu$ l	400 $\mu$ l
Mini Mag, Extraction reagents	NucliSens, BioMerieux Marcy l'Etoile, France	manual	Magnetic silica	RNA/ DNA	50 $\mu$ l	1ml
NucliSens Manuel Isolation kit	NucliSens, BioMerieux Marcy l'Etoile, France	manual	Silica	RNA/ DNA	50 $\mu$ l	1ml
Nuclisens automated isolation kit	NucliSens, BioMerieux Marcy l'Etoile, France	automated	Silica membrane	RNA/ DNA	50 $\mu$ l	1ml

Table 2. Expression of U1A mRNA using PreTect HPV-Proofer

Method	Hitrate (n/n)	Average RFU	Range of RFU ratio
RNAquous-Micro M48 Biorobot.	49/50	2,37	2.11 - 2.61
Magattract RNA cell mini kit	79/80	2,56	2.11 - 2.94
RNeasy mini kit	100/100	2,36	2.11 - 2.52
Perfect RNA, Eucaryotic, Mini	49/50	2,36	1,53 - 2.83
RNA-spin	50/50	2,25	1.79 - 2.46
Versagene, RNA cell kit	59/60	2,41	1.62 - 2.83
Mini Mag Extraction reagents	114/116	2,48	1.60 - 2.85
NucliSens Manuel Isolation kit	59/60	2,67	1.81 - 2.77
Nuclisens automated isolation kit	147/150	3,90	1.94 - 5.12

Figure 1A: Nucleic acid extraction by spin columns.



Figure 1B: Nucleic acid extraction by magnetic silica beads.

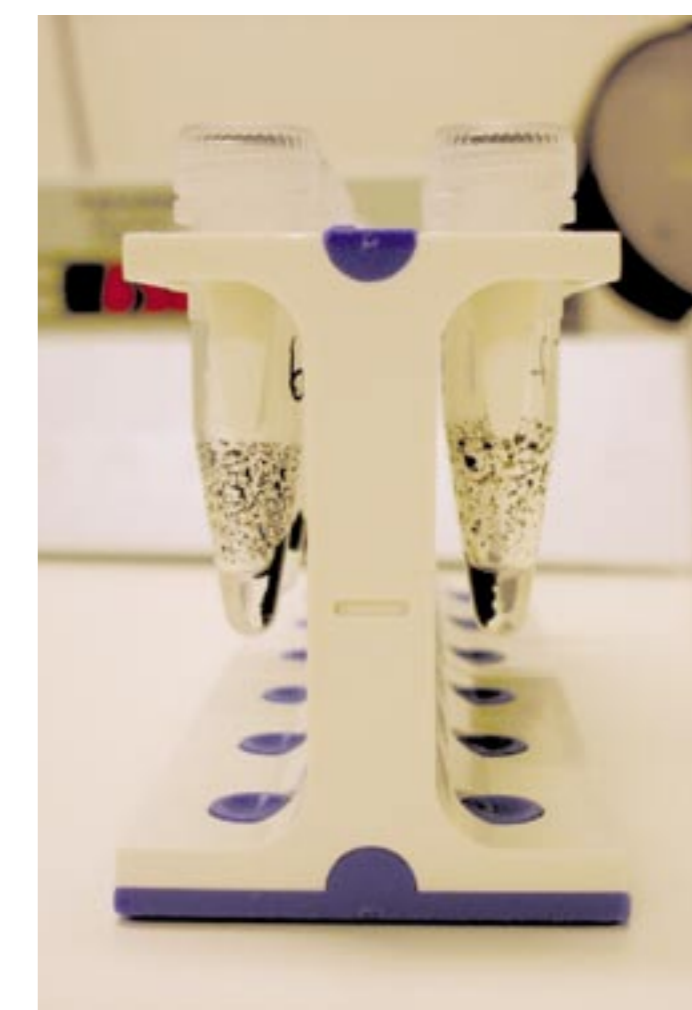


Figure 2. Principle of NASBA

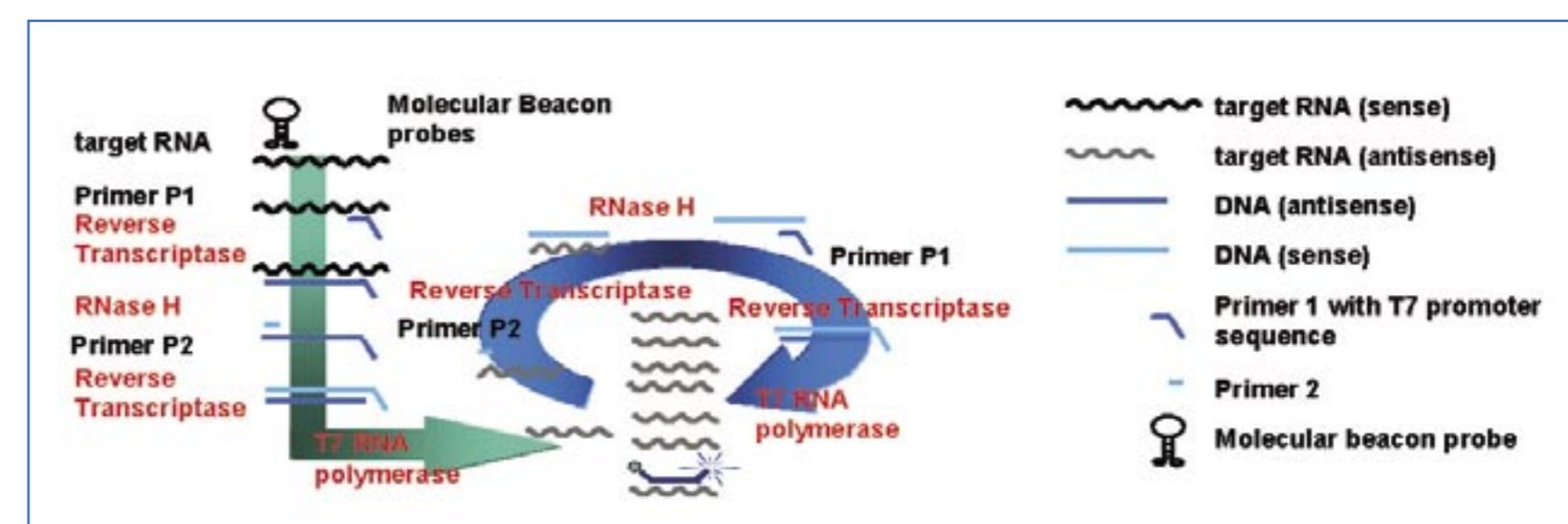
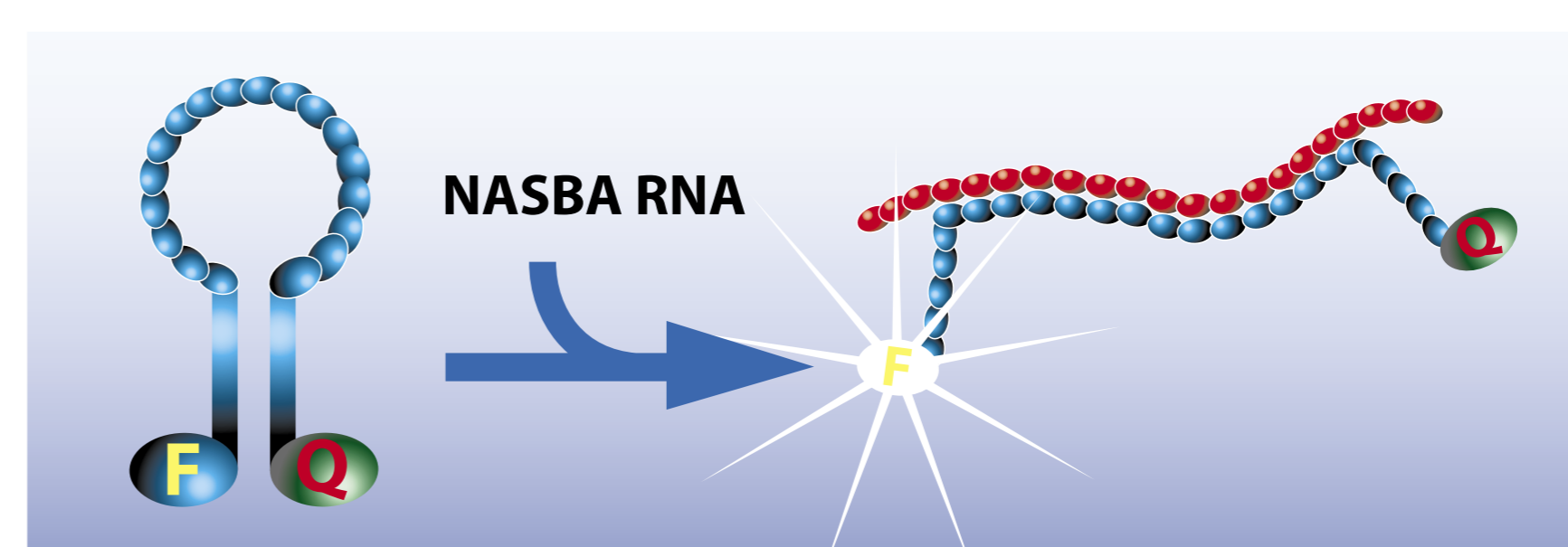
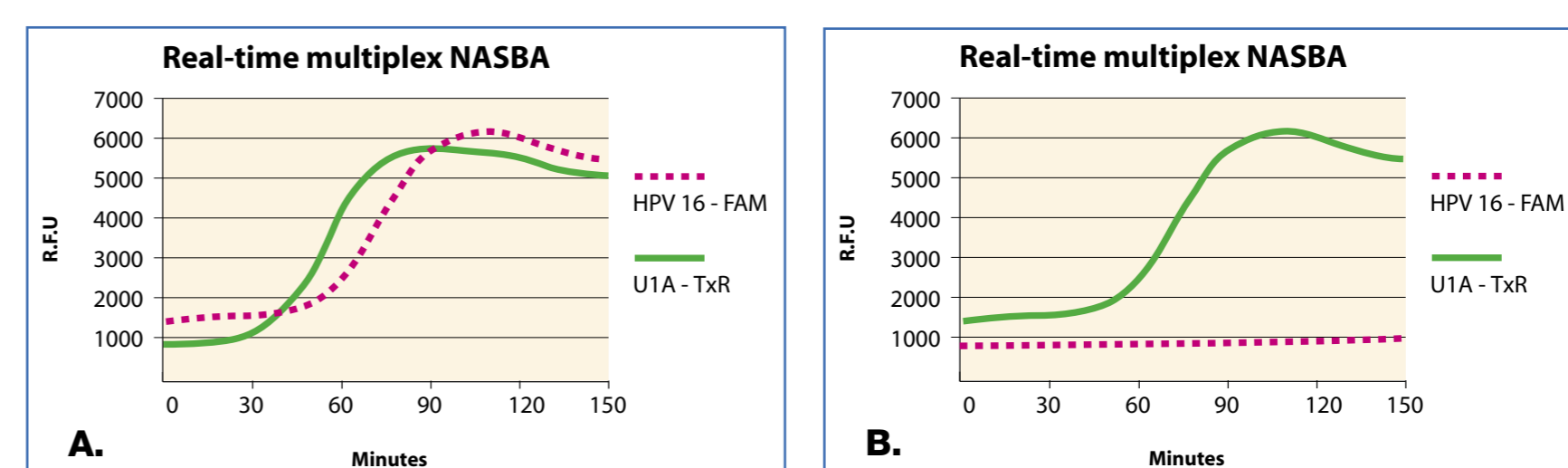


Figure 3. Principle of Molecular Beacon



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.

Figure 4. 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.

## References

(1) Analytical performance of the PreTect HPV-Proofer assay for detection of E6/E7 mRNA expression and typing of 5 high-risk HPVs. Tor Molden, Trine Nordstrom, Linda Kristiansen, Irene Kraus, Frank Karlsen and Hanne Skomedal 22nd International Papillomavirus Conference and Clinical Workshop 2005. Vancouver, Canada.