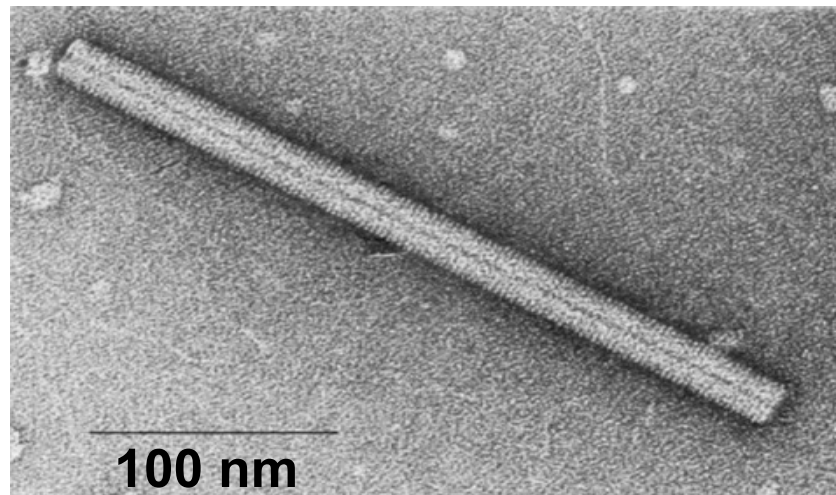


# Use of Tomato Mosaic Virus (ToMV) as Internal Positive Control (IPC) in different RT-PCR settings

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[www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)

# What is an Internal Positive Control (IPC) good for ?

**will control inhibition**

will control pre-analytical steps (transport, ...) ?

will control storage of sample before analysis ?

**will control nucleic acid extraction of sample**

**will control reverse transcription (RNA viruses)**

**will control PCR-efficacy**

will be detected with adequate features ?

# Concept of IPCs

control the amplification - but do not influence it

2 general principles:

***competitive IPC***

very similar to target

same primer

restricted:

control for one system

similar amplicon lengths

***„less“ competitive IPC***

different from target

different primer

universal:

control for different PCR systems

amplicon length adequate

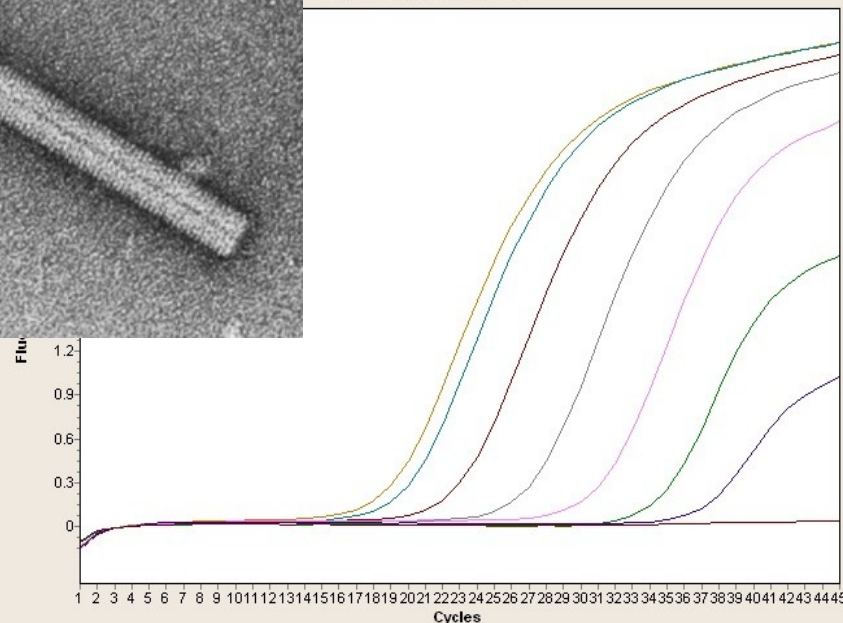
spike sample with optimal type/amount of IPC

# ToMV – tomato mosaic virus

**Features:** ss-RNA virus, (+) polarity, 6390 nt  
very stable, well characterized  
can be easily propagated and purified  
non-pathogenic for humans  
is obtained in high amounts (one g of virus !)



Amplification Curves



# samples tested with ToMV-IPC

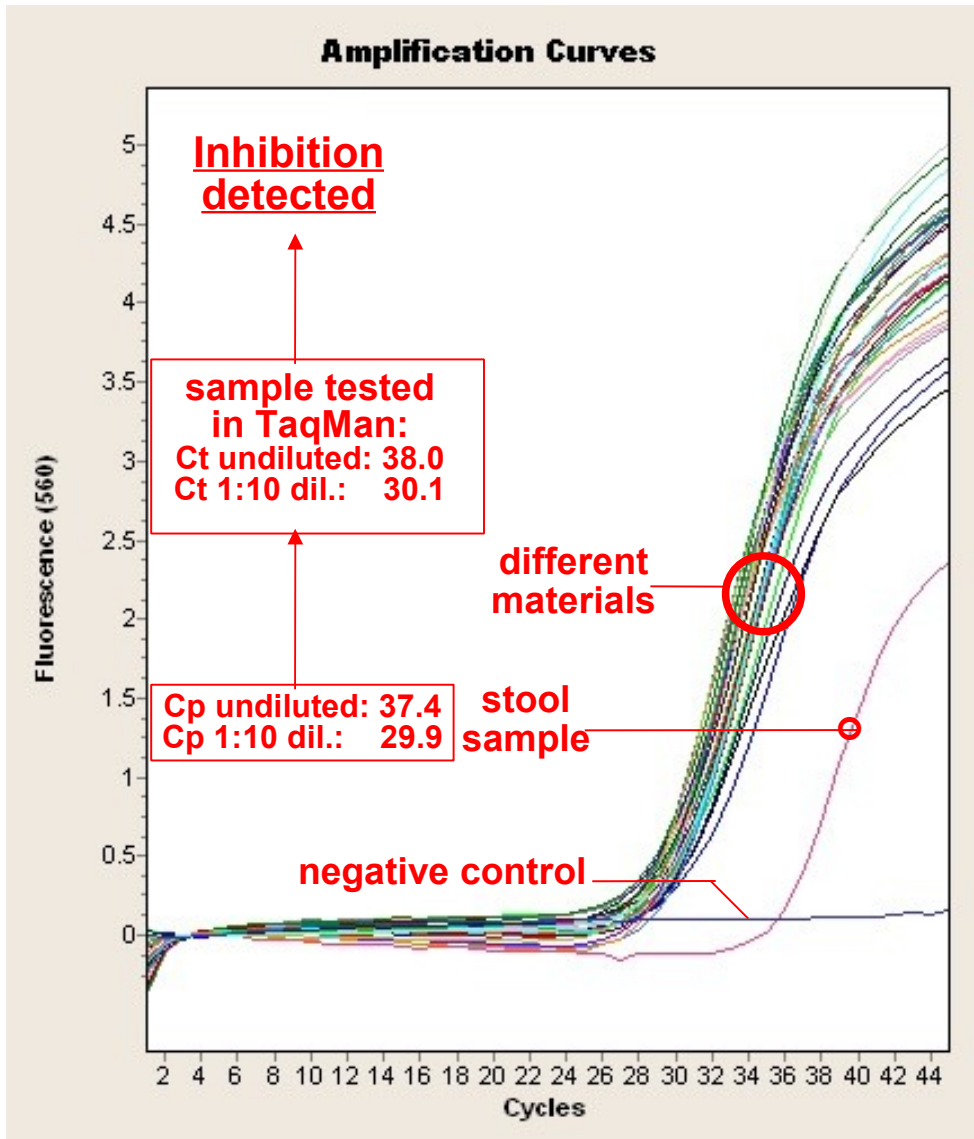


LMU

| <u>sample</u>            | <u>numbers tested</u> |
|--------------------------|-----------------------|
| stool                    | 101                   |
| stool (1:10 diluted)     | 88                    |
| PBL                      | 74                    |
| pharyngeal wash          | 66                    |
| bronchoalveolar lavage   | 28                    |
| urine                    | 19                    |
| biopsies                 | 14                    |
| ENTA                     | 12                    |
| swabs                    | 11                    |
| plasma                   | 9                     |
| aspirate                 | 7                     |
| sputum                   | 7                     |
| serum                    | 5                     |
| secretion                | 5                     |
| bone marrow              | 1                     |
| liquor                   | 1                     |
| cell culture supernatant | 1                     |

**Total: n = 361 patients**  
and 88 stool samples

# LightCycler I – ToMV IPC added to different specimen



ToMV-IPCs  $10^{-3}$  dilution

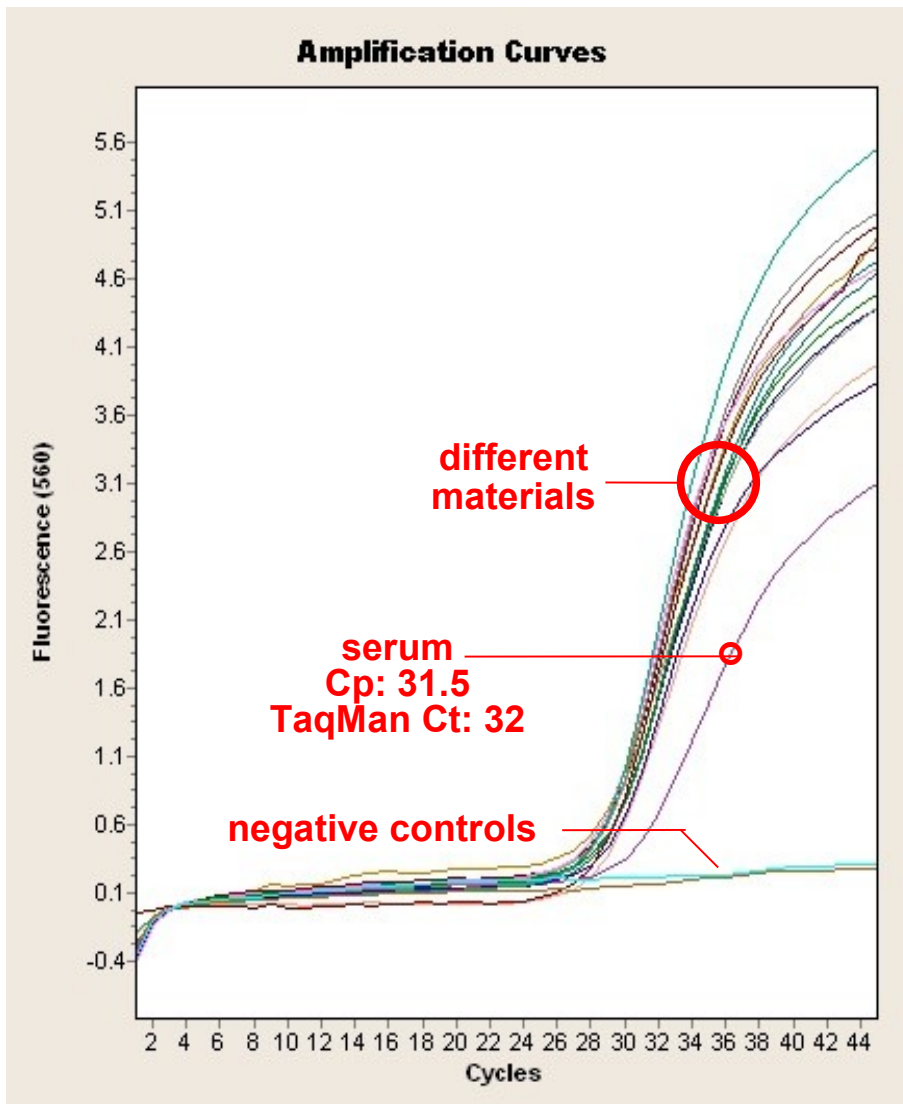
extraction of samples:  
High Pure Viral Nucleic  
Acid Kit  
(Roche, spin column)

RT: random primer +  
ToMV-specific primer

PCR: ToMV-specific  
primer

Hex-Tamra ToMV  
specific probe

# LightCycler II - ToMV IPC added to different specimen



**ToMV-IPCs  $10^{-3}$  dilution**

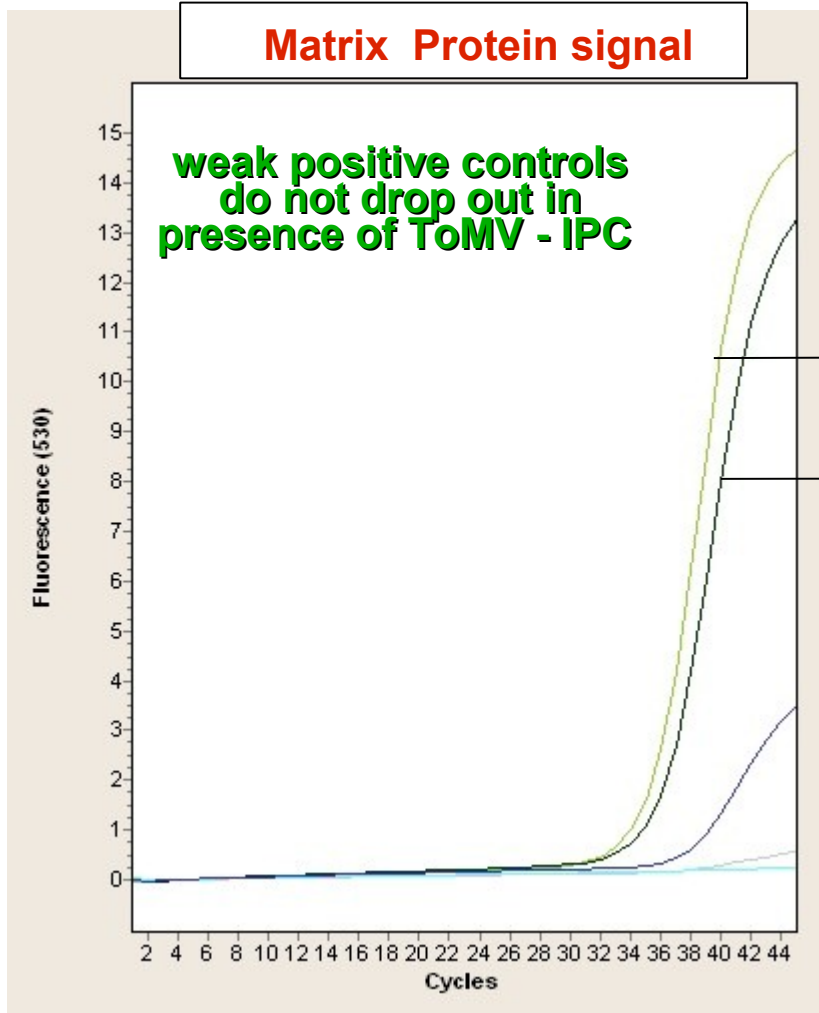
**extraction:  
High Pure Viral Nucleic  
Acid Kit (Roche, spin  
column)**

**RT: random primer +  
ToMV-specific primer**

**PCR: ToMV-specific  
primer**

**Hex-Tamra ToMV-  
specific probe**

# Influenza A virus "quantified" controls with ToMV-IPC



4 weak positive influenza A virus controls are positive (Cp values similar to routine diagnostic without ToMV-IPC)

purified RNAs

control influenza  $10^{-6}$  ✓

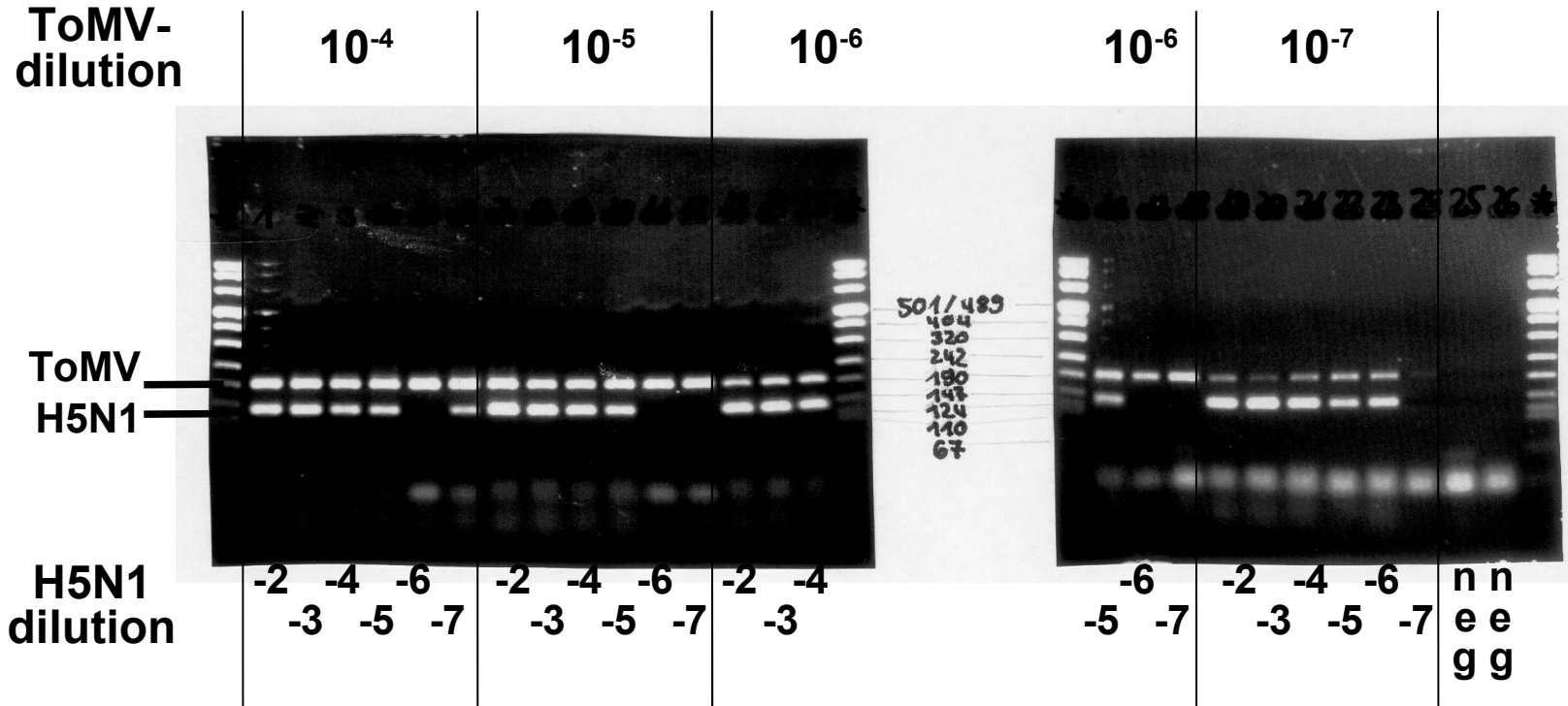
control H5N1  $10^{-3}$  ✓

control H5N1  $10^{-4}$  ✓

control influenza  $10^{-7}$  ✓

# PCR LightCycler amplicons

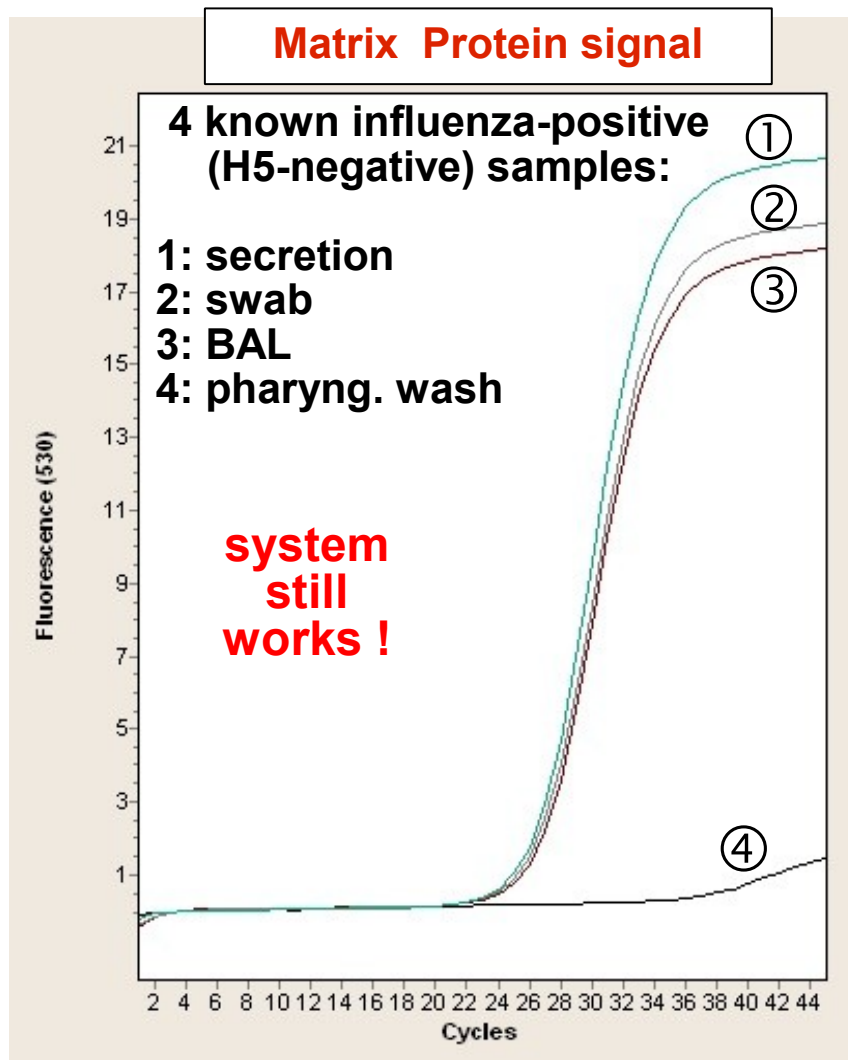
ToMV - IPC coamplified with influenza (H5N1)



**2 specific amplicons with correct size**

# LightCycler – now a bit more complicated

detection of influenza A virus in **clinical specimen**, manual extr.



## existing system: LC-detection

1 pair of primers for influenza virus  
matrix protein + m-probe

1 pair of primers for influenza  
virus type H5 + H5-specific Probe

**we add: ToMV-IPC**

RT-mix contains:

MP-F

MP-R

EP-F

EP-R

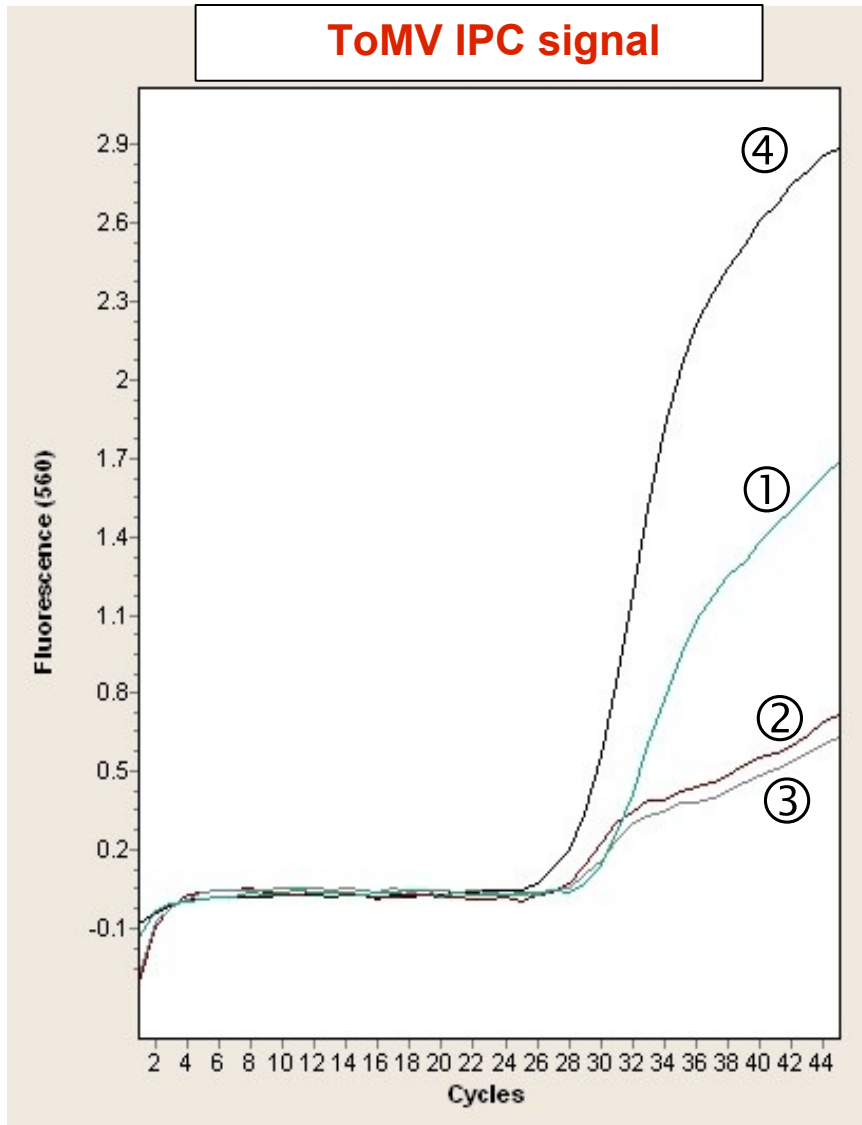
**TMVC01**

**TMVC02**

**random primer**

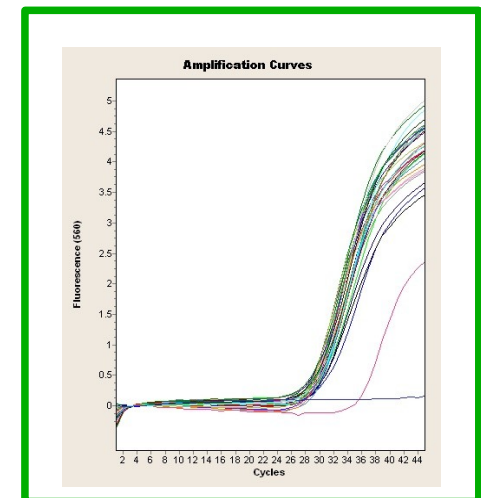
in PCR-mix: probes for matrix-protein  
(Fam) and ToMV (Hex)

# LightCycler – now a bit more complicated detection of influenza A virus in **clinical specimen**

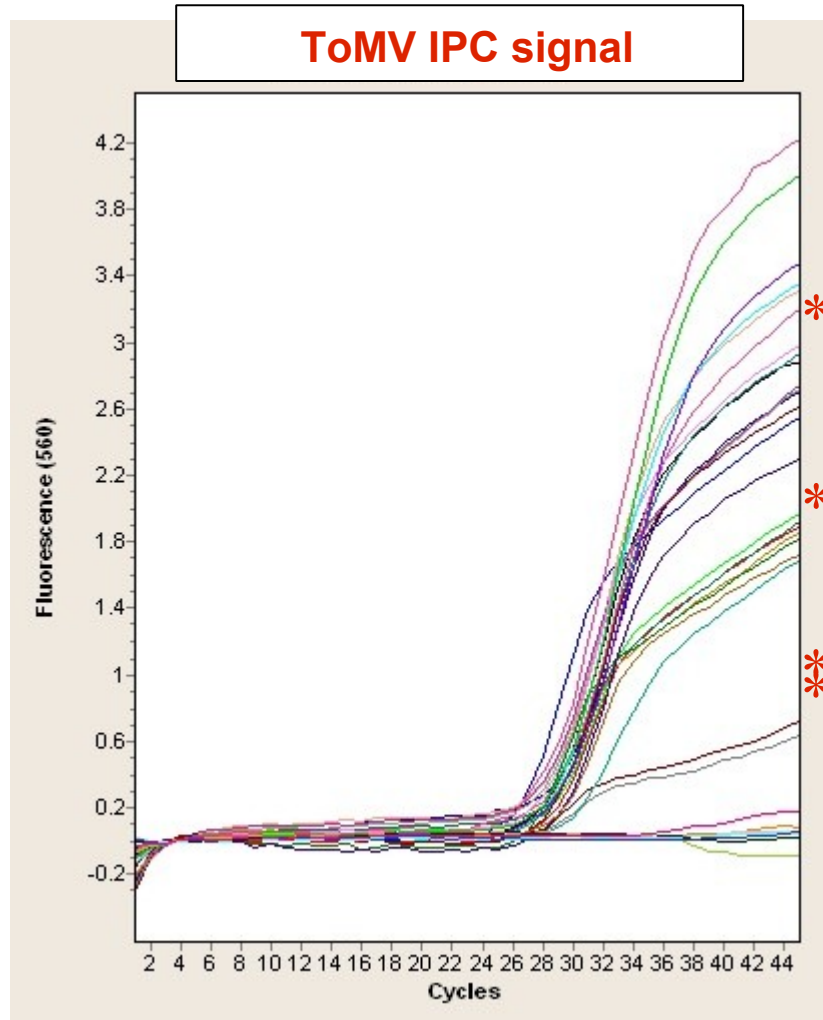


samples 1,2 and 3 contain  
high copy numbers of  
influenza A virus genomes,  
sample 4 only minor amounts

**competition situation**  
influenza A virus - ToMV IPC ?



# Influenza A virus and ToMV-IPC – patient samples



## ToMV-IPCs $10^{-3}$ dilution

extraction of samples: High Pure  
Viral Nucleic Acid Kit  
(Roche, spin column)

RT-mix contains:

MP-F

MP-R

EP-F

EP-R

**TMVC01**

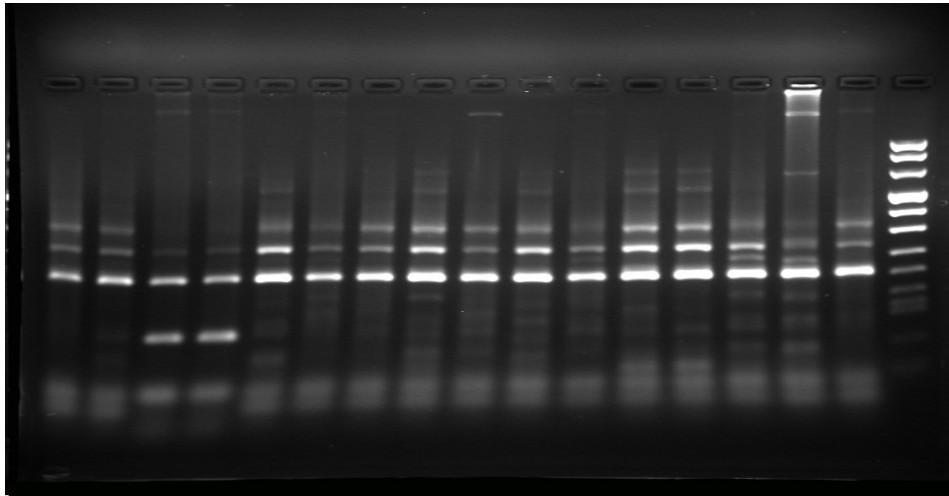
**TMVC02**

**random primer**

in PCR-mix: probes for matrix-  
protein (Fam) and ToMV (Hex)

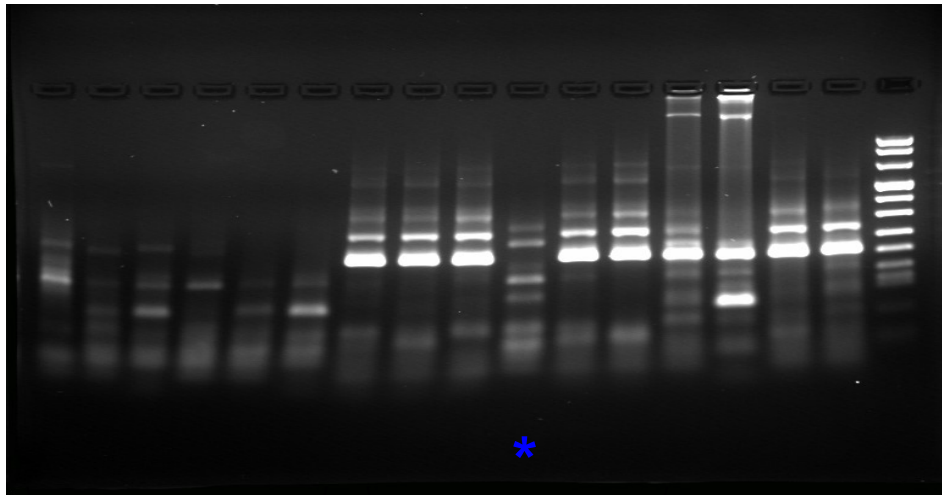
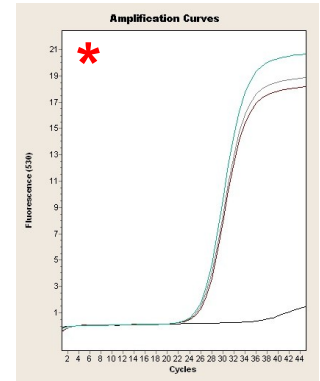
|                          |   |
|--------------------------|---|
| samples: pharyngeal wash | 9 |
| bronchoalv. lavage       | 4 |
| aspirates                | 2 |
| secretions               | 2 |
| sputum                   | 2 |
| swab                     | 1 |

# Patient specimen influenza A virus and ToMV-IPC:



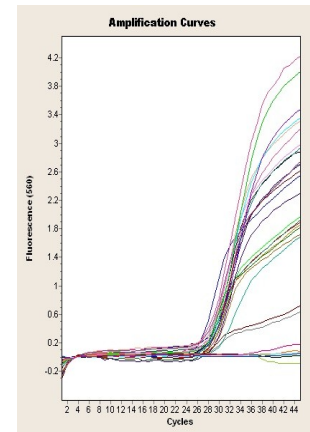
— ToMV  
— influenza matrix prot.

\* \* (\* )

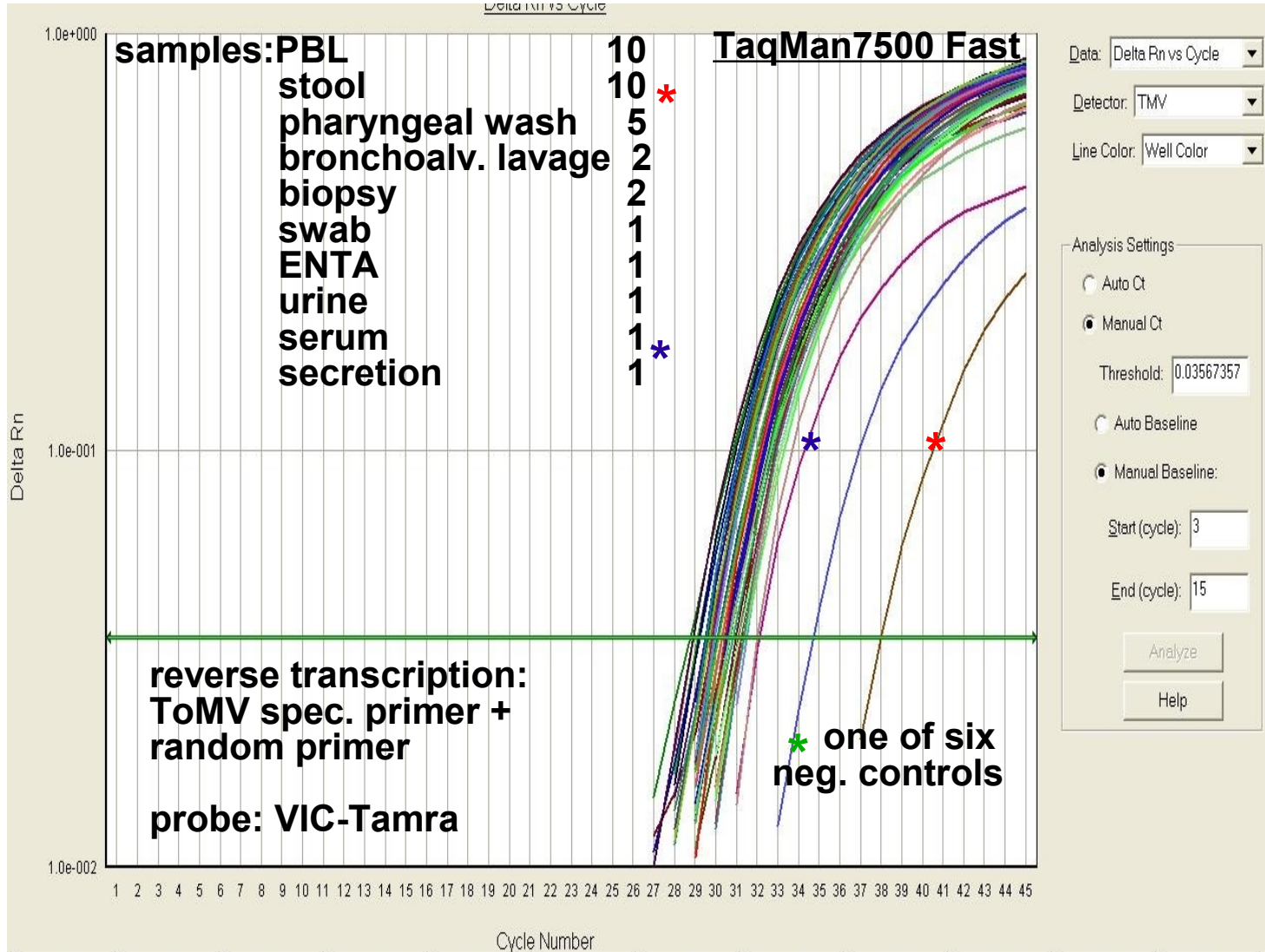


— ToMV  
— influenza matrix prot.

infl. controls: no ToMV    extraction controls

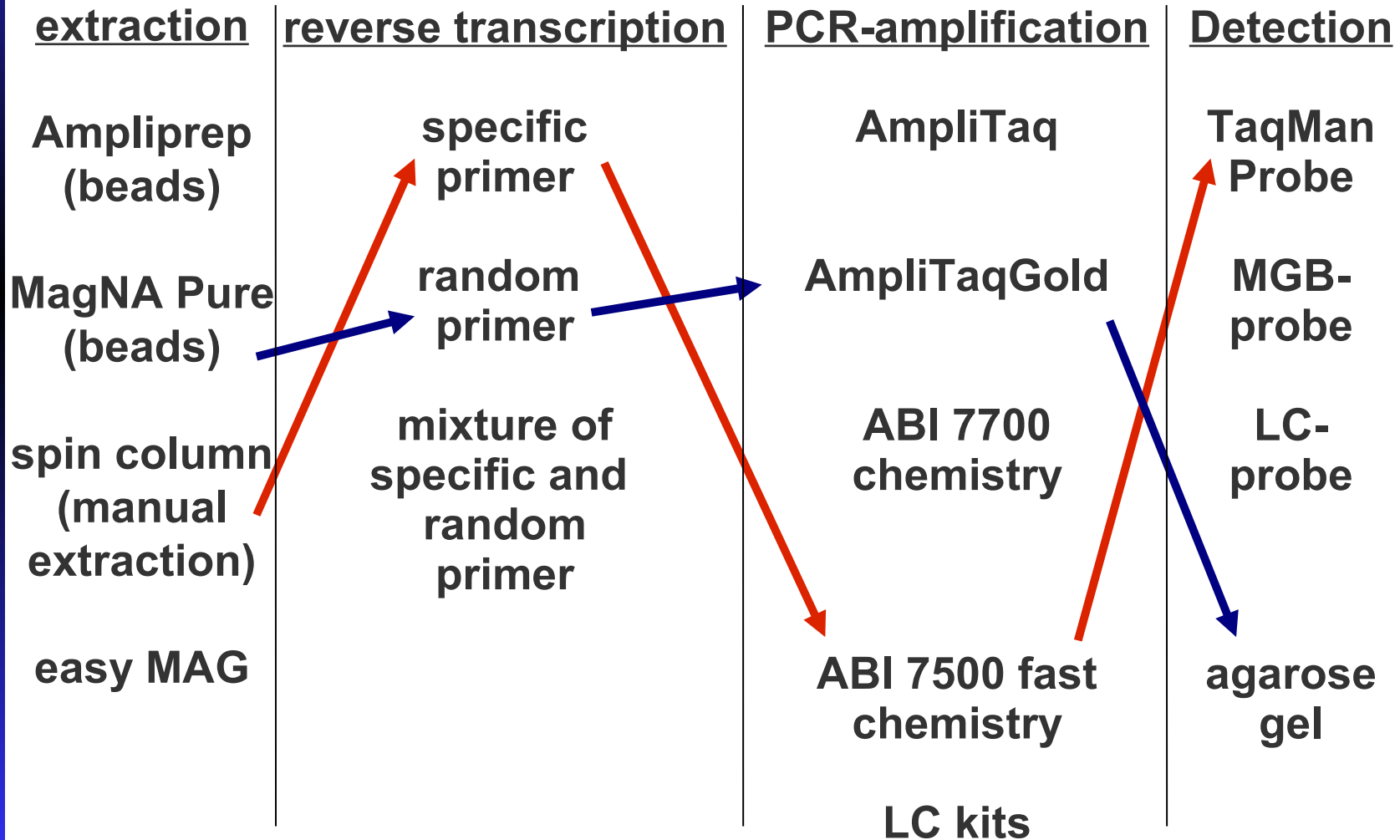


# MagNA Pure extracted ToMV – IPC



# ToMV as IPC

now it is really complicated



# What do we look for ?

*serum, plasma, liquor*  
more or less cell free

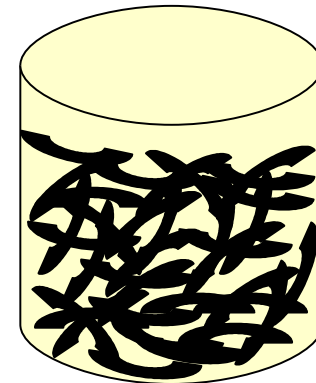
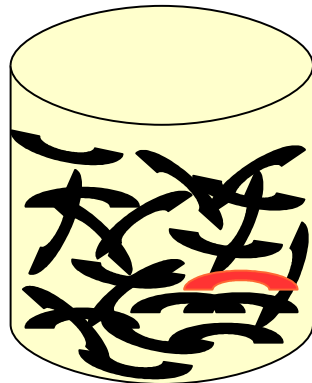
*biopsies, stool, PBL*  
different number/types of cells

one cell contains:

cytoplasmat. m-RNA  $7 \times 10^5$   
cytoplasmic ribosomes  $4 \times 10^6$   
cytoplasmic t-RNA  $6 \times 10^7$   
nuclear precursor r-RNA  $6 \times 10^4$   
heterogen. nuclear RNA  $2 \times 10^5$   
and about 3 pg DNA

example EBV:

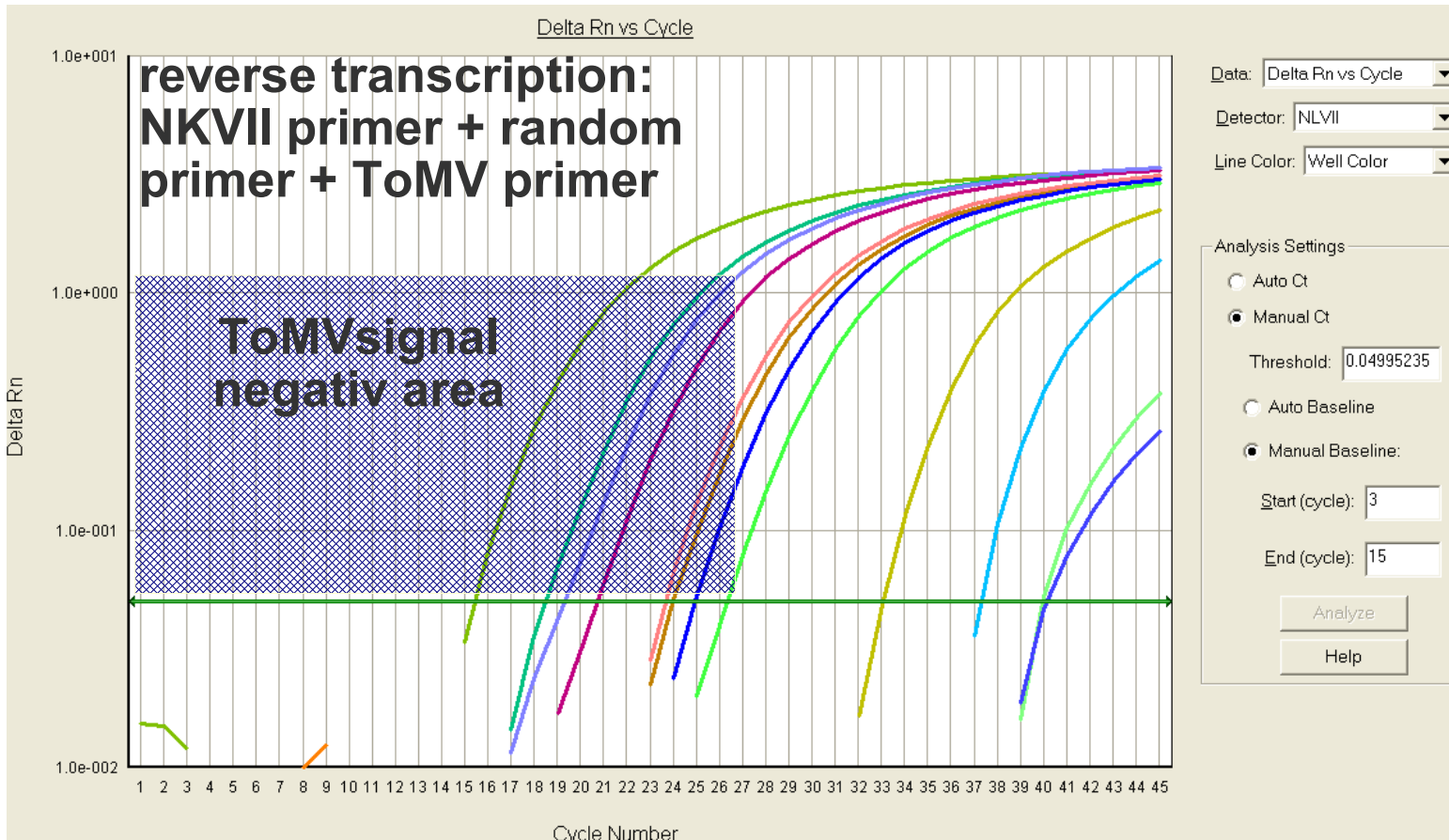
1-10 EBV-infected cells  
within 100.000 B-lymphocytes  
(latency)



# Detection of Norovirus (genotype II) in presence of ToMV - IPC

31 stool samples and two biopsies

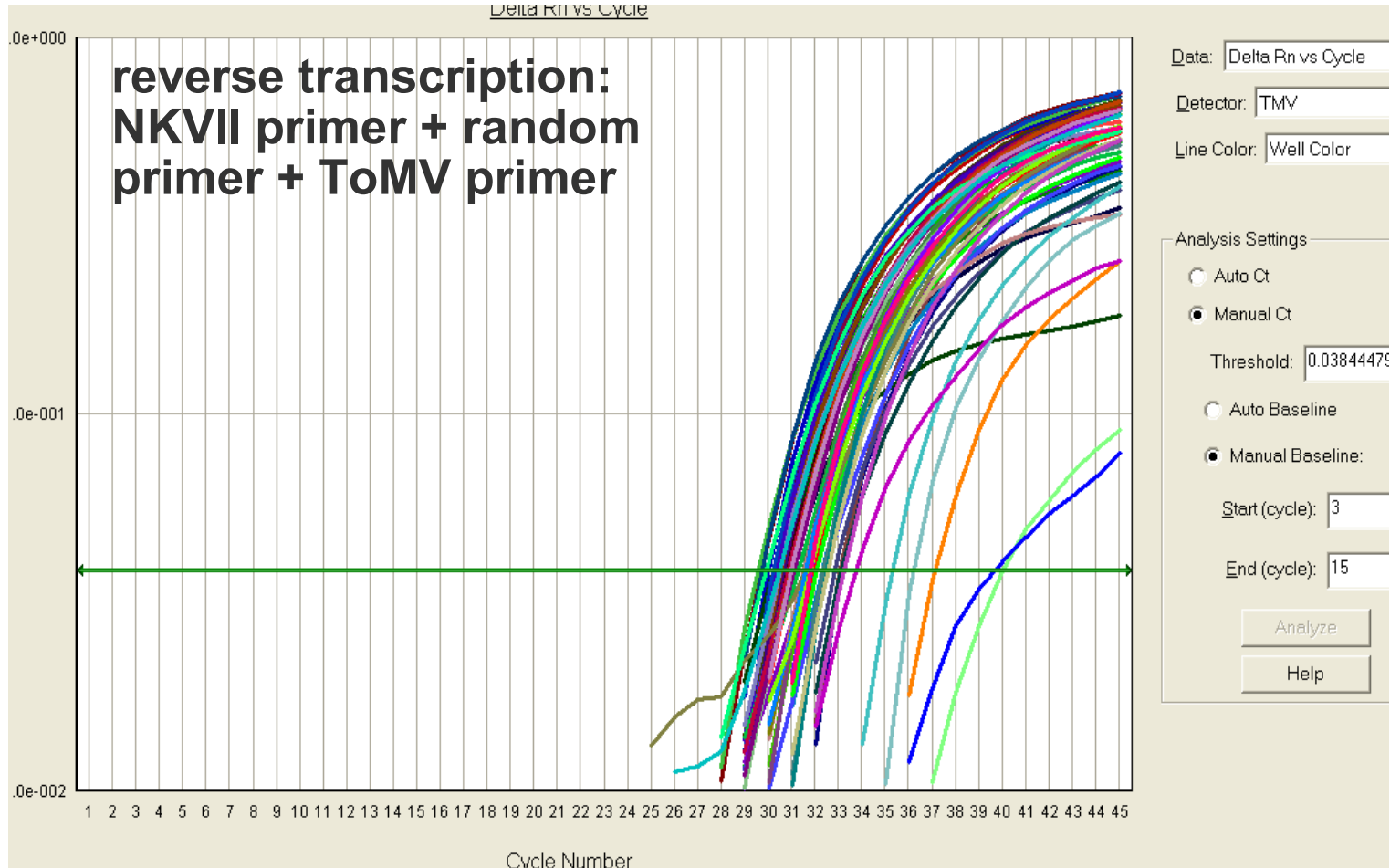
readout: NLV II signal



**Ct-values for NLV-signals about 1-2 Ct higher when ToMV is present**

# Detection of Norovirus (genotype II) in presence of ToMV - IPC

31 stool samples and two biopsies  
readout: ToMV - IPC signal



**both biopsies inhibited, 4 stool samples inhibited (2 samples still inhibited in 1:10 dilution), large amounts of NLV: IPC will drop out.**

# Summary

**tomato mosaic virus is a useful Internal Positive RNA Control**

**the applicable quantitative range of IPC to be added is small (within one log); “to high”: weak positive samples drop out, “to low”: signal not stable particular in cell rich specimen**

**the percentage of clearly inhibited samples (shift Cp/Ct >3) was low 7/361 (2%), flat curves/minor Cp/Ct shifts are frequent and not easily explained**

**patient samples perform definitively different from „clean“ control materials, robustness of system must be tested with appropriate specimen**

**ToMV-IPC works in ABI TaqMan, LC, conventional PCR (gel), does not work in combination with Norovirus type I**