



H1N1 rapid detection assay

- Fast determination of Influenza A subtype H1N1
- Based on Rapid Amplification Hybridization (RAH) technology
- Including optimized reagents for RNA extraction, RT-PCR, PCR amplification and final detection on a lateral flow stripe (LFS)

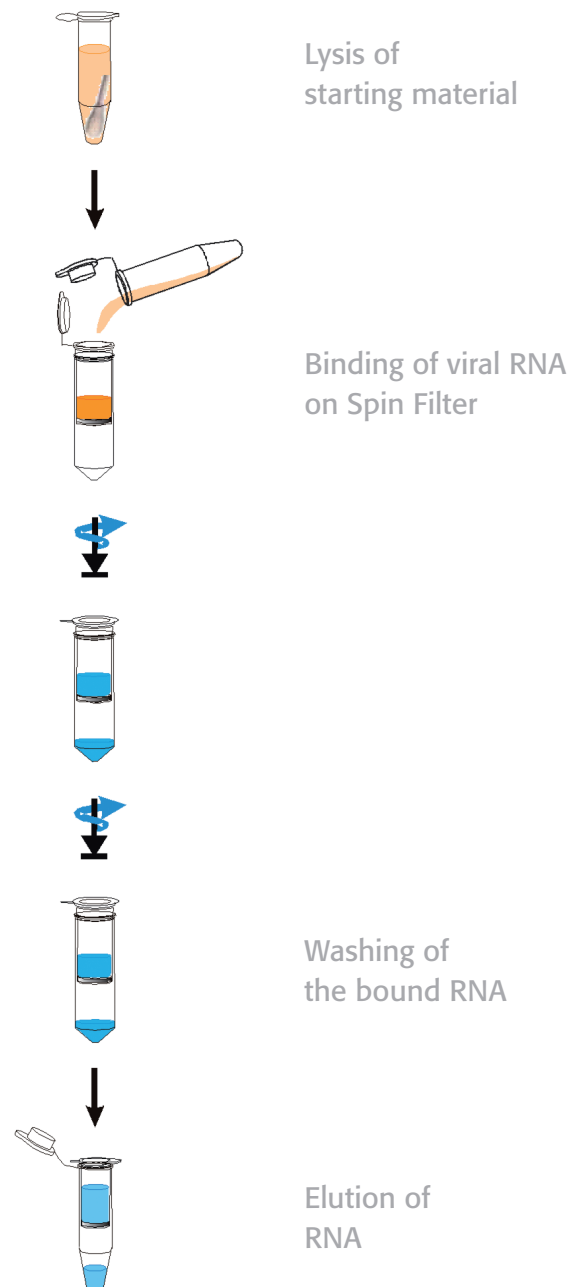
rapidSTRIPE H1N1 Assay

The Influenza A Virus subtype H1N1 is a new mutation of flu virus, which results from a genetically combination – a so called fourfold combination – of two virus lines of the swine flu (R.J. Garten, C.T. Davis et al., Science 325: 5937; 197-201; 2009). The rapidSTRIPE H1N1 Stripe Assay has been developed as a new diagnostic platform for very fast, efficient and specific detection of the Influenza A H1N1. Therefore the modular structure of the rapidSTRIPE Detection System combines all steps of molecular diagnostics

- 1. Isolation of nucleic acids
- 2. RT-PCR and cDNA amplification
- 3. Detection of target nucleic acids

1. Isolation of Virus RNA from H1N1

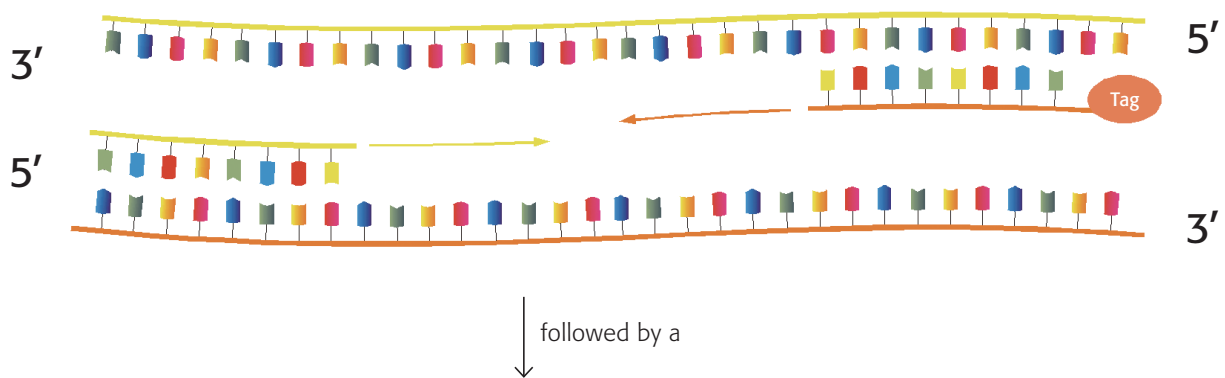
- The extraction procedure is based on a new patented technology for isolation of viral RNA from tracheal or nasal swabs.
- After sample lysis, using a very stringent lysis buffer system, the RNA is bound to a Spin Filter surface by a novel binding buffer, following washing and the final elution of the bound RNA.



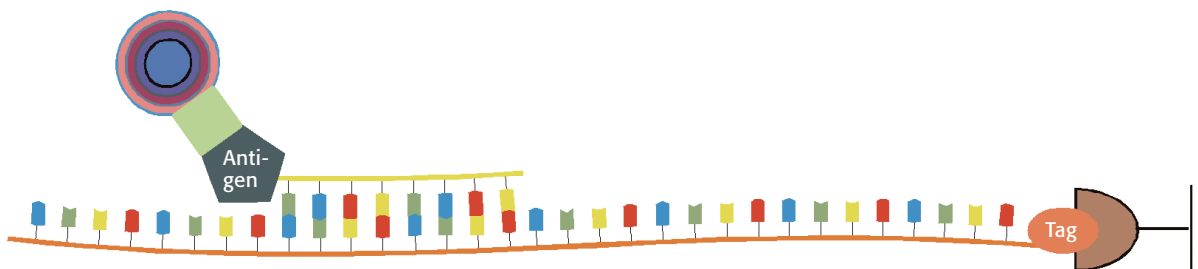
2. Rapid Amplification and Hybridization (RAH)

- Subsequent to the nucleic acid isolation the cDNA synthesis is followed using a RT enzyme and H1N1 specific primers, which are also included into the rapidSTRIPE H1N1 Stripe Assay.
- Afterwards the synthesized cDNA will be specific amplified by rapidPCR technology on AlphaSC® or SpeedCycler² of Analytik Jena | bio solutions.
- Besides amplification on standard PCR thermal cyclers, like FlexCycler is also possible.
- The amplification is in the following combined with a hybridization reaction using a A/H1N1 virus specific probe.
- Both reactions are proceeded in the same reaction vessel, without adding further reagents.
- This reaction format allows thus a specific determination of the A/H1N1 RNA and avoids false negative results because of a mispriming.

PCR amplification of the synthesized cDNA with a forward primer and a tag-labeled reverse primer.



Denaturation of the PCR fragments and hybridization of a sequence specific, antigen labeled probe.



3. Detection of H1N1 on LFS

- The hybridization of the amplification products with an antigen labeled probe is done by using the combined amplification/hybridization time and temperature protocol, whereby the sequence of the probe is complementary to the tag-labeled DNA strand.
- The detection of the specific amplification reaction is carried out on a lateral flow stripe (LFS) by transfer of the amplification mixture on the test stripe without any further manipulation.

- The A H1N1 virus cDNA specific fragments will be conjugated with antibody coated gold particles by antigen-antibody interaction. A flow buffer is used to move the gold particles to a coated region on the LFS, for further binding of the tag. Thus the gold particles are accumulated at this part of the lateral flow stripe and becomes visible in a violet test line.

- Besides a second control line, as conjugate control will give answers about the quality of the LFS.

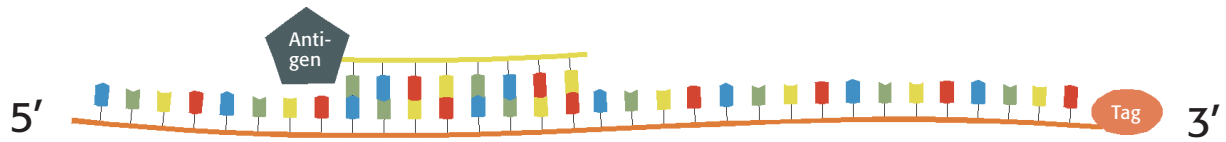
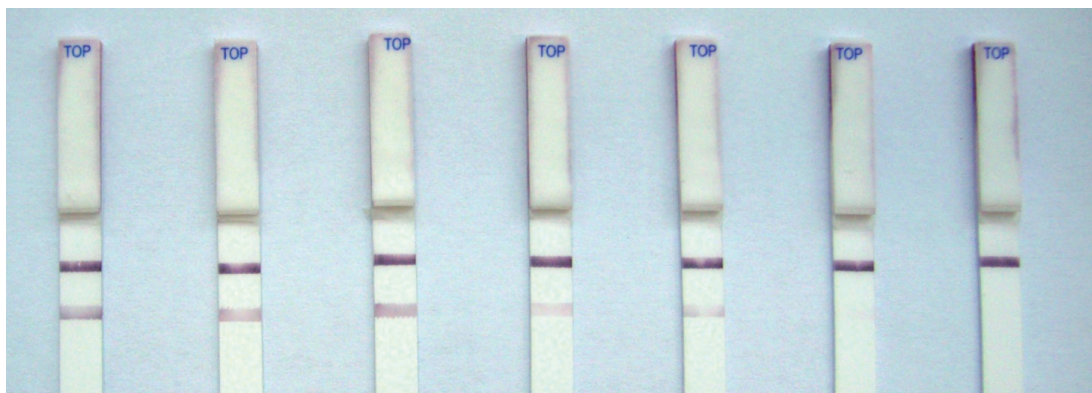
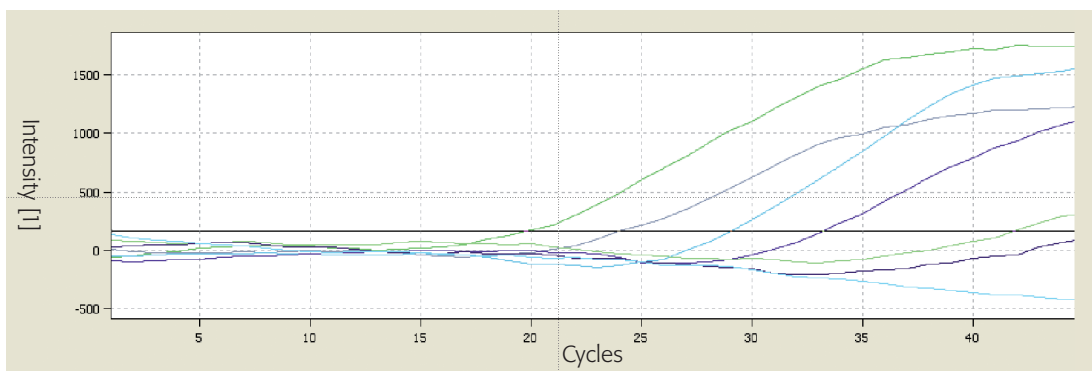


Fig. 1: Detection on lateral flow stripe (LFS)



Sample dilution	Non diluted	1 : 10	1 : 100	1 : 1.000	1 : 10.000	1 : 100.000	Negative control
ct values (qPCR)	19,9	23,8	29,2	33,3	41,8	No ct	No ct
Lateral flow stripe (LFS)	Positive	Positive	Positive	Positive	Positive	Negative	Negative

Fig. 2: Determination of ct values using real time PCR



Specifications

Time for isolation:
 Lysis: 15 min
 Extraction: approx. 10 min

Time for RT-PCR:
 approx. 50 min

Time for RAH:
 AlphaSC® or SpeedCycler2:
 approx. 50 min

Starting material:
 Tracheal or nasal swab

Sensitivity:
 Comparable to qPCR

Publisher:
Analytik Jena AG
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October 2009, Analytik Jena AG
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