Nucleic acid-based multiplex technique for the detection and differentiation of avian influenza A virus subtypes H5, H7 and H9

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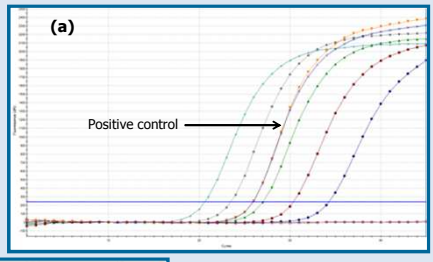
Introduction

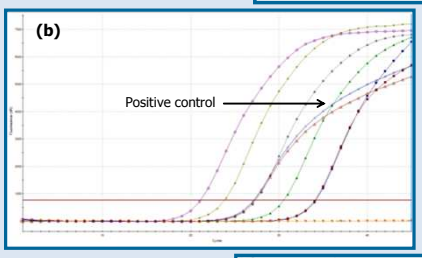
Beginning of 2015 an increasing incidence of avian influenza outbreaks in commercial poultry was observed worldwide and highly pathogenic avian influenza virus got once again into the focus of public attention.

Influenza A viruses belong to the notifiable animal diseases and express the surface antigens haemagglutinin (H) and neuraminidase (N) which can occur in various variants. Especially subtypes H5, H7, H9 are of high epidemiological importance.

Figure 1: Real-time PCR data for SureFast® Influenza A H5/H7/H9 4plex on the Agilent Mx3005P

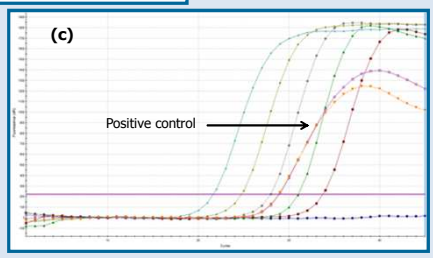
(a) Standard curve (10^1 - 10^5 DNA copies/µl) and positive control for the detection of Influenza A **H5** in the **FAM-channel**

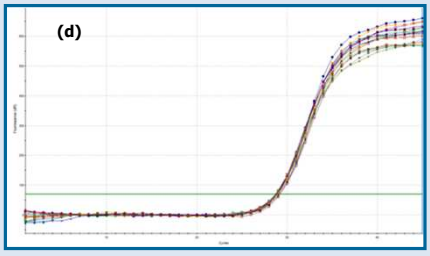




(b) Standard curve (10^1 - 10^5 DNA copies/µl) and positive control for the detection of Influenza A **H7** in the **Rox-channel**

(c) Standard curve (10^1 - 10^5 DNA copies/µl) and positive control for the detection of Influenza A **H9** in the **Cy5-channel**





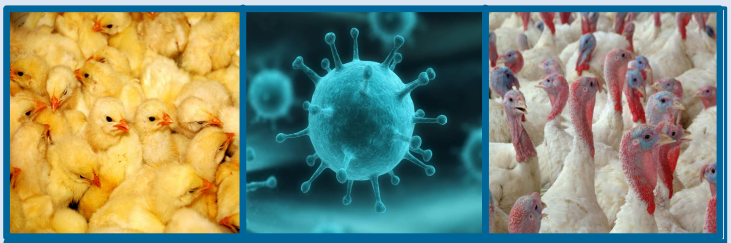
(d) Internal amplification control (**IAC**) in the **VIC-channel**

Material and Methods

After isolation of RNA by using SureFast® PREP DNA/RNA Virus, detection was carried out as "One Step RT-qPCR" system with SureFast® Influenza A H5/H7/H9 (both CONGEN, Berlin).

This multiplex PCR test is applicable on a variety of real-time PCR instruments equipped for simultaneous detection of four fluorescence emissions at 522 nm, 553 nm, 610 nm and 670 nm. Moreover, the test contains an internal control RNA (extraction and/or amplification control).

Table 1: Validation data of specificity testing for SureFast® Influenza A H5/H7/H9 4plex



Sample (selection)	Influenza A H5	Influenza A H7	Influenza A H9
Adenovirus	negative	negative	negative
Astrovirus	negative	negative	negative
Coronavirus	negative	negative	negative
Enterovirus	negative	negative	negative
Enterovirus	negative	negative	negative
Hepatitis A	negative	negative	negative
Herpes simplex virus 1	negative	negative	negative
Herpes simplex virus 2	negative	negative	negative
Influenza A H1N1/Mallard/Germany	negative	negative	negative
Influenza A H2N9/Mallard/Germany	negative	negative	negative
Influenza A H3N8/Mallard/Germany	negative	negative	negative
Influenza A H4N6/Mallard/Germany	negative	negative	negative
Influenza A H5N1/Whooper swan/Germany	**positive**	negative	negative
Influenza A H5N2/Mallard/Netherlands	**positive**	negative	negative
Influenza A H5LPN3/Mallard/Germany	**positive**	negative	negative
Influenza A H5N8/Black-headed Gull/Sweden	**positive**	negative	negative
Influenza A H6N1/Turkey/Germany	negative	negative	negative
Influenza A H7N1/Bewick's Swan/Netherlands	negative	**positive**	negative
Influenza A H7N2/Mallard/ Netherlands	negative	**positive**	negative
Influenza A H7N3/Mallard/ Netherlands	negative	**positive**	negative
Influenza A H7N4/Eurasian Wigeon/Netherlands	negative	**positive**	negative
Influenza A H7LPN7/Turkey/Germany	negative	**positive**	negative
Influenza A H7N8/Red Knot/Sweden	negative	**positive**	negative
Influenza A H8N4/Turkey/Ontario	negative	negative	negative
Influenza A H9N2/Turkey/Germany	negative	negative	**positive**
Influenza A H9N2/Bewick's Swan/Netherlands	negative	negative	**positive**
Influenza A H9N3/Black-headed Gull/ Republic of Georgia	negative	negative	**positive**
Influenza A H10N7/Mallard/Germany	negative	negative	negative
Influenza A H11N6/Duck/England	negative	negative	negative
Influenza A H12N5/Duck/Alberta	negative	negative	negative
Influenza A H13N2/Mediterranean Gull/Germany	negative	negative	negative
Influenza A H14N5/Mallard/Gurjev	negative	negative	negative
Influenza A H15N9/Shearwater/Westaustralia	negative	negative	negative
Influenza A H16N3/Herring Gull/Germany	negative	negative	negative
Influenza B	negative	negative	negative
Sapovirus	negative	negative	negative
Metapneumovirus	negative	negative	negative
MS2-Phage	negative	negative	negative
Norovirus GI.3	negative	negative	negative
Norovirus GII.4	negative	negative	negative
Parainfluenza virus Type 1	negative	negative	negative
Parainfluenza virus Type 2	negative	negative	negative
Parainfluenza virus Type 4b	negative	negative	negative
Rhinovirus Group A	negative	negative	negative
Rotavirus	negative	negative	negative

Results

Analysis of 113 influenza A strains belonging to subtypes H5 (n=51), H7 (n=38) and H9 (n=24) showed inclusivity of 100% Beside the target analytes also non-target sequences were examined (n≥180), whereby an exclusivity value of 100% was determined. Extracts of results are shown in Tab. 1. Depending on the matrix, processing grade, RNA preparation and RNA content the theoretical detection limit was ≤ 25 RNA-copies.

Conclusion

Variants H5, H7, H9 cause significant economic damage in the affected production plants and have a high zoonotic potential.

Thus, targeted monitoring of different influenza A virus subtypes is gaining increasingly in importance.

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