Supporting Information

Dual Recognition Element Lateral Flow Assay (DRELFA)-Towards Multiplex Strain Specific Influenza Virus Detection

Thao T. Le,[a], Pengxiang Chang[b,] Donald J. Benton[c], John W. McCauley[c], Munir Iqbal[b], and Anthony E.G. Cass*[a]

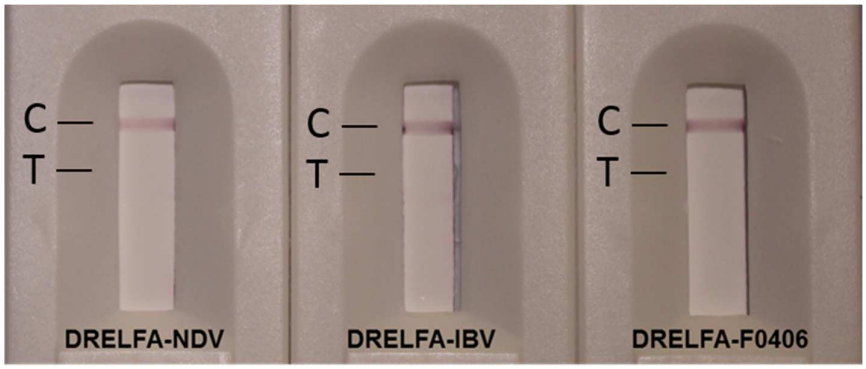
Aptamer sequences

Sequences of the RNA aptamers and corresponding DNA templates used

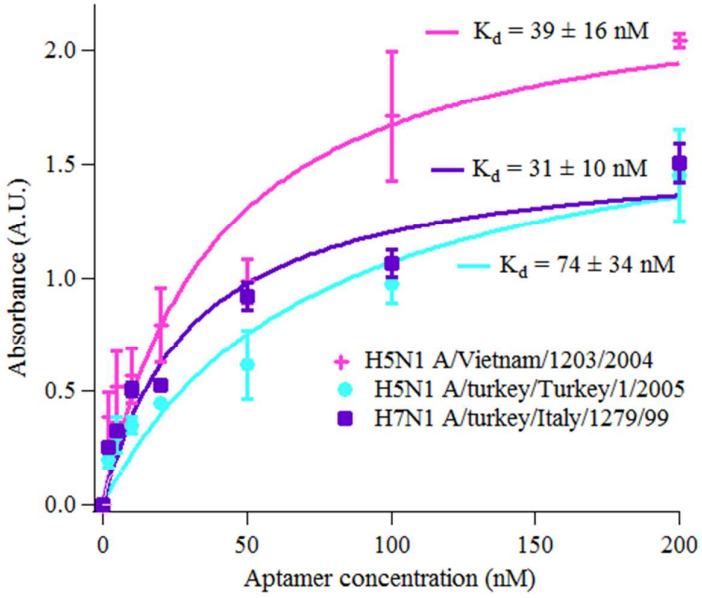
SI Table: Oligonucleotide sequences

Name	Role	Sequence
RNAH3P07Apt	RNA aptamer for A/Panama/99/2007 (H3N2)	GGGAGAAUUCCGACCAGAAGGGUUAGCAGUCGGCAUGCGGUAC AGACAGACCUUUCCUCUCUCCUUCCUCUUCU
ApH3P07	DNA template for the RNA aptamer selected against A/Panama/99/2007 (H3N2)	TCTAATACGACTCACTATAGGGAGAATTCCGACCAGAAGGGTTAG CAGTCGGCATGCGGTACAGACAGACCTTTCCTCTCTCCTTCCTCTTC T
ForH3P07	Forward primer for ApH3P07	TCTAATACGACTCACTATAGGGAGAATTCCGACCAGAAG
RevH3P07	Reverse primer for ApH3P07	AGAAGAGGAAGGAGAGAGGAAAGG
ApH3P07Linker	Linker with a biotin for the RNA aptamer against A/Panama/99/2007 (H3N2)	5'-biotin-TEG-AGAAGAGGAAGGAGAGAGG
RNAH5VN04Apt	RNA aptamer for Vietnam/1203/2004 (H5N1)	GGAGCUCAGCCUUCACUGCGAGAUUGUUCUGACCGAGUUGCCU AGCAGGGCAACCGCUGGAACUUGAAGUCGGUAAUGCGAGCGGA AAGCCUUGGCACCACGGUCGGAUCCAC
ApH5VN04	DNA template for the RNA aptamer	TCTAATACGACTCACTATAGGAGCTCAGCCTTCACTGCGAGATTGT TCTGACCGAGTTGCCTAGCAGGGCAACCGCTGGAACTTGAAGTCG GTAATGCGAGCGGAAAGCCTTGGCA CCACGGTCGGATCCAC
ForH5VN04	Forward primer for ApH5VN04	TCTAATACGACTCACTATAGGAGCTCAGCCTTCACTGC
RevH5VN04	Reverse primer for ApH5VN04	GTGGATCCGACCGTGGTGCC
ApH3VN04Linker	Linker with a biotin for the RNA aptamer against	5' Biotin-TEG-GTGGATCCGACCGTGGTGCC
DNAH9N2Apt	DNA aptamer against purified hemagglutinin H9 protein[1]	5' Biotin-TEG-GCTGCAATACTCATGGACAGGGGCCGCGCCTGGTCGGTTGGGTGG GTGGCGCCCGGGACGGTCTGGAGTACGACCCTGAA

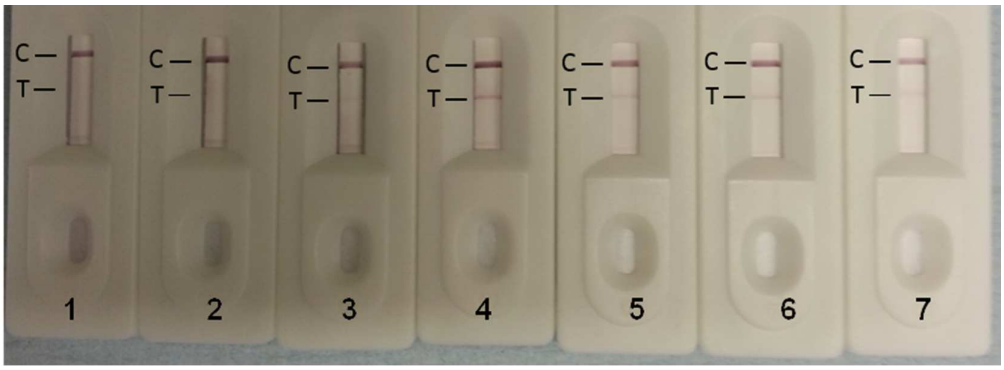
Additional Data



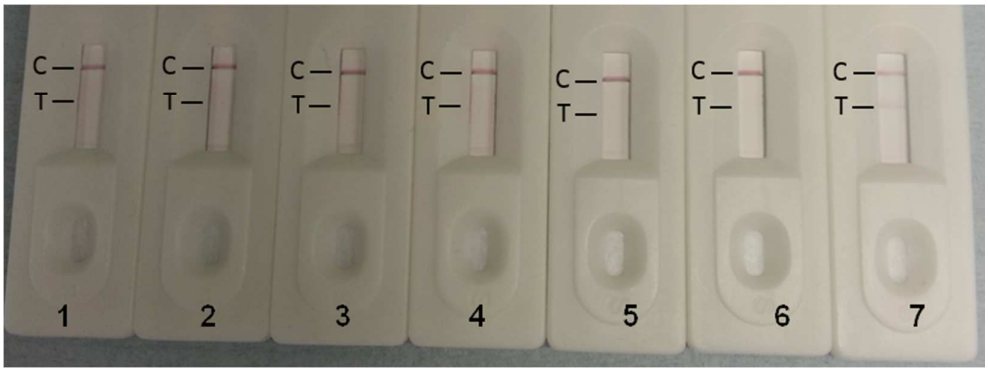
SI1 Figure: Cross-reactivity test of the DRELFA format with 3 viruses (Newcastle disease virus (NDV), infectious bronchitis virus (IBV) and an influenza B virus, B/Florida/04/2006 (F0406) at a concentration of $3x10^8$ virus particles per sample. All three viruses showed only the control line (C) with no visual detectable signal on the test line (T). The measurements were performed on a DRELFA comprising a reported RNA aptamer selected against an influenza A virus strain A/Panama/2007/99 (H3N2) and an anti-hemagglutinin H3 antibody.



SI2 Figure: Binding of a reported RNA aptamer selected against the influenza A strain A/Vietnam/1203/2004 (H5N1) to whole virus particles of 3 different viruses: Vietnam/1203/2004 (H5N1); A/turkey/Turkey/1/2005 (H5N1) and A/turkey/Italy/1279/99 (H7N1). Binding data were obtained using Enzyme Linked Oligo Nucleotide Assays (ELONAs).



SI3 Figure 1: H5N1-aptamer DRELFAs on different virus samples (1: A/Panama/2007/99 (H3N2); 2: A/Udorn/307/72 (H3N2); 3: A/Aichi/2/68 (H3N2); 4: A/Vietnam/1203/2004 (H5N1); 5: A/turkey/Turkey/1/2005 (H5N1); 6: A/turkey/Italy/1279/99 (H7N1); 7: A/chicken/Pakistan/UDL/2008 (H9N2)). All viruses used in this study were inactivated vaccine strains and were generated using reverse genetic technique carrying HA and NA gene of indicated strains and internal genes from a laboratory strain of A/Puerto Rico/8/1934 (H1N1) virus. The HA genes of high pathogenicity H5N1 viruses also lack polybasic cleavage site. The DRELFA format comprised a reported RNA aptamer selected against influenza A virus strain A/Vietnam/1203/2004 (H5N1) with the respective hemagglutinins monoclonal antibodies; anti-hemagglutinin H3, anti-hemagglutinin H5, anti-hemagglutinin H7 or anti-hemagglutinin H9. All samples contained 10^8 virus particles as measured by nanoparticle tracking analysis



SI3 Figure 2: H9N2-aptamer DRELFAs on virus samples (1: A/Panama/2007/99 (H3N2); 2: A/Udorn/307/72 (H3N2); 3: A/Aichi/2/68 (H3N2); 4: A/turkey/Turkey/1/2005 (H5N1); 5: A/turkey/Italy/1279/99 (H7N1); 6: A/Vietnam/1203/2004 (H5N1); 7: A/chicken/Pakistan/UDL/2008 (H9N2)). The DRELFA format comprised a DNA aptamer selected against influenza A virus strain A/chicken/Pakistan/UDL/2008 (H9N2) with the respective anti hemagglutinin (H3, H5, H7 or H9) antibodies. All samples contained 10^8 virus particles.

References

(1) Zhang, Y., Yu, Z., Jiang, F., Fu, P., Shen, J., Wu, W. and Li, J., *PLoS ONE*, **2015**, 10, e0123060.