

Digital and Quantifiable Rapid Diagnostic Test (RDT) for Avian Influenza Using Rapid Diagnostic Reader (RDR)

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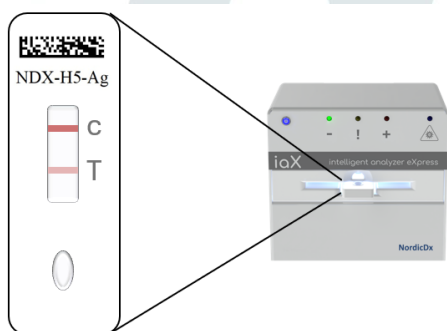
1. Avian Influenza Virus (AIV)

The UK Health Security Agency has raised the H5N1 (Bird Flu) outbreak to Level 4 (out of 6). This level indicates the possibility of persistent mammalian outbreaks with increasing human cases and limited human-to-human transmission, primarily associated with animal exposures.

By implementing screening and early detection using a Rapid Diagnostic Reader (RDR), we can effectively reduce these risks.

2. iaX- intelligent analyze eXpress

The iaX machine is an innovative RDR designed for the rapid and quantitative diagnosis of a wide range of Rapid Diagnostic Tests (RDT) in the form of Lateral Flow Assays (LFA). It is integrated with a cloud-based data storage system, where all the results are automatically uploaded and stored securely. The tests are “onboarded” on the RDR, a term used to describe that the iaX is programmed to analyze a test and give quantitative results. The RDR can read very weak test lines that are not visible for the naked eye, and can also read fluorescent RDTs, thereby enhancing the sensitivity and specificity of the tests.



3. AIV-LFA test Instruction for Use:

- 3.1. Collect swab samples from poultry Laryngeal tracheal or Cloacal Discharge.
- 3.2. Insert the swab into the buffer tube, stir and squeeze.
- 3.3. Pipette 100µL of the sample/buffer mixture onto the test well.
- 3.4. Insert the test into the RDR and read the result in 8 minutes and not after 20 minutes. Stronger positive samples may develop before 8 minutes.

4. AIV Onboarding on the iaX machine:

- 4.1. A 2-fold dilution series with the following concentrations was prepared:

Dilution Series	Concentrations (ng/mL)
1:1	40
1:2	20
1:4	10
1:8	5
1:16	2.5
1:32	1.25
1:64	0.625

- 4.2. Unique barcodes were generated for each test.
- 4.3. Each dilution was read 5 times. 8 negative control samples were read, 5 times each. 4 blank tests (no buffer added) were read 3 times each. The average and standard deviation for each dilution was calculated.

Dilution Series	Concentrations (ng/mL)	Average RI	SD
1:1	40	20.07	0.24
1:2	20	10.29	0.56
1:4	10	5.19	0.10
1:8	5	2.12	0.13
1:16	2.5	0.72	0.06
1:32	1.25	0.67	0.04
1:64	0.625	0.54	0.03
Negative Control	0	0.46	0.05
Blank	0	0.56	0.06

- 4.4. LOD was considered at RI = 0.65 ± 0.031.

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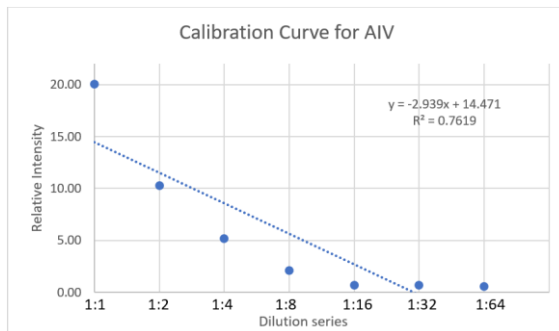


Figure 1. Calibration Curve for Avian Flu (H5 Antigen), Using AIV Positive Control Dilutions.

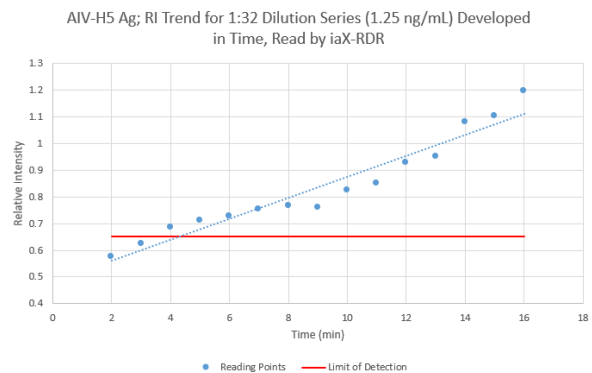


Figure 3. AIV-H5 Ag, dilution series 1:32. The average RI readings are recorded from 2 minutes, every minute. The red line indicates the threshold (LOD).

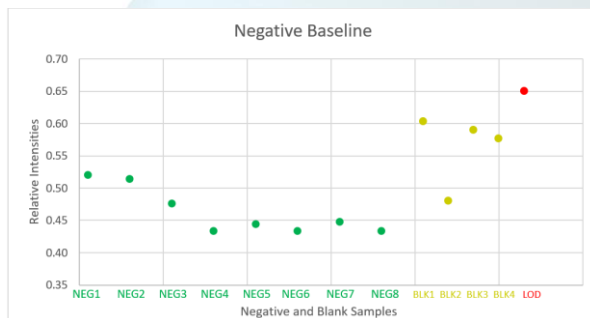


Figure 2. Negative Baseline. Negative and Blank Samples are highlighted by green and yellow respectively. LOD is illustrated in red.

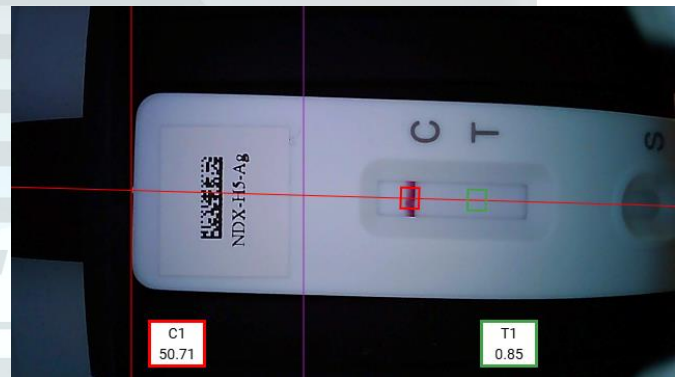


Figure 4. AIV-H5 Ag, dilution series 1:32. As shown in the figure, the machine can detect the line, even though it is not visible.

5. Development Time

The weakest positive was developed again to study the incubation time. The RDR can detect weak positives after 4 minutes on average, even though a visible line can't be detected by the naked eye. For an added safety margin, it is suggested that tests may be read using the iaX-RDR after approximately 8 minutes.