Update on LAMP Project

OFFLU Technical Meeting, 4-5 April 2012
Royal Holloway, UK
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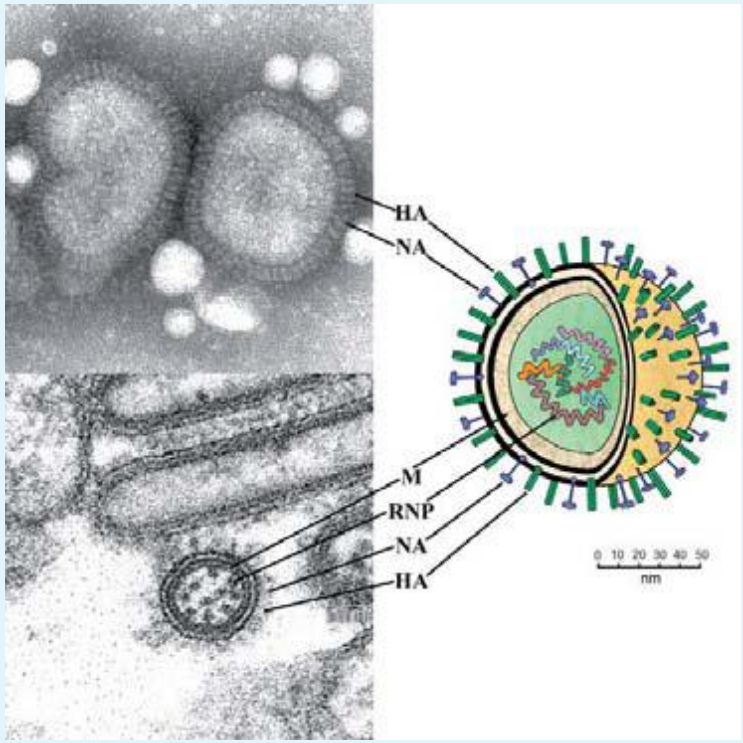
Early and Rapid Diagnosis of Influenza

Need for (rapid) tests with the following properties:

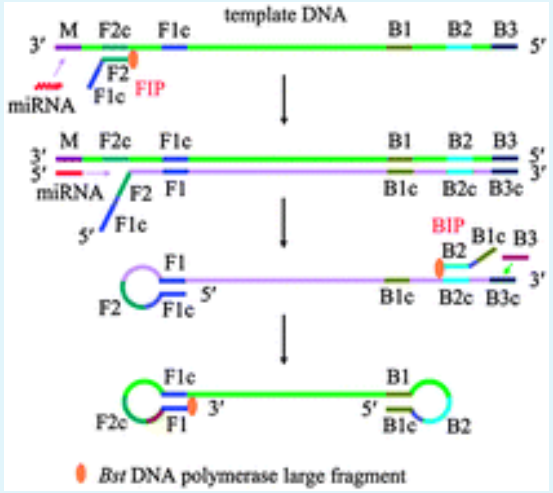
- basic, but of high quality
- easy handling and interpretation (under BSL-1)
- independant of sophisticated equipment
- safe
- feasible, affordable
- robust (storage)
- availability: easy to procure
- validated (sensitive, specific)
- Minimum chance for cross-contamination
- Possibe to use in developing countries

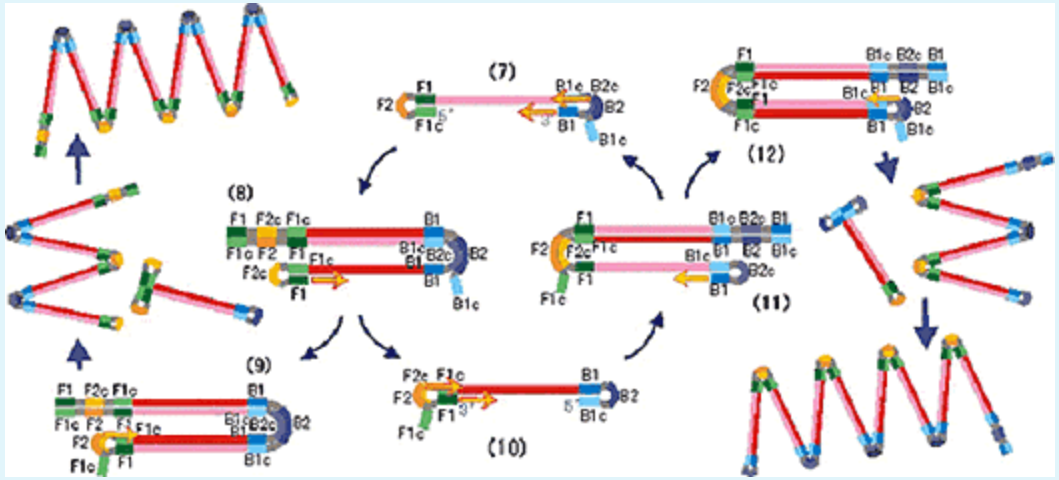


Friedrich-Loeffler-Institut

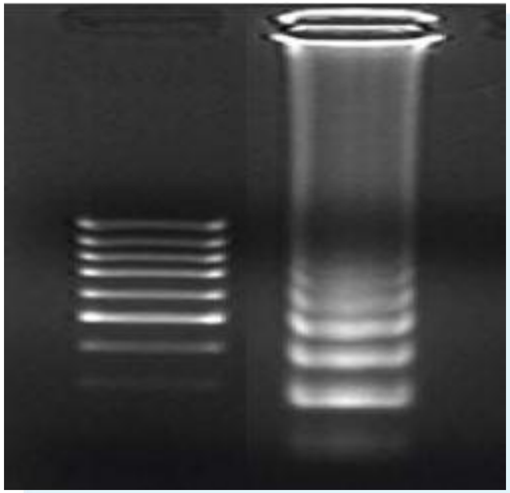
LAMP (Loop mediated Isothermal AMPlification)

- One version of polymerase amplification methods
- Rapid molecular method
- Set of six primers recognizing eight distinct regions on the target gene
- Result within one hour
- Isothermal conditions (waterbath / heat block)
- Single tube





http://loopamp.eiken.co.jp/e/lamp/principle.html

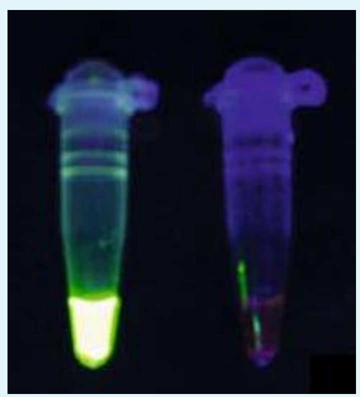


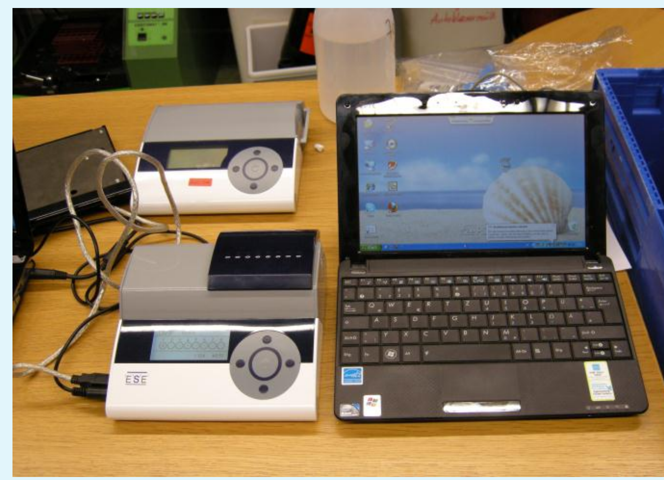
Agarose gel Analysis

Visual Turbidity

Normal Light

UV Lamp

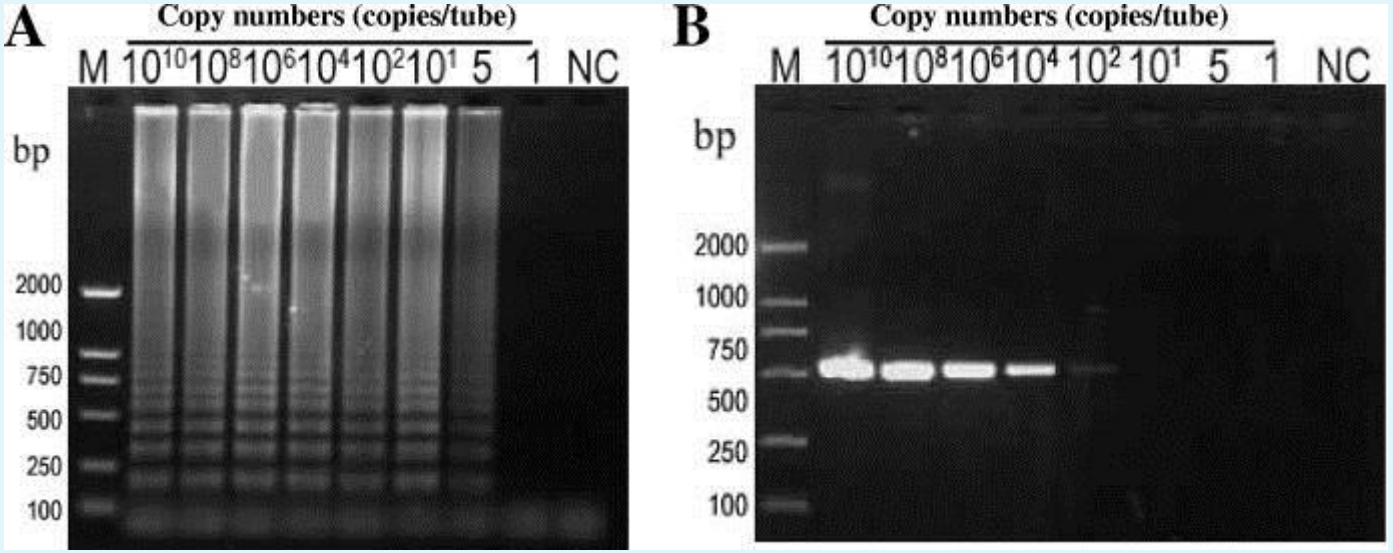




LAMP developed in 2000

(Notomi et al.(2000) Nucleic Acids Res 28: E63)

Comparative sensitivities of the LAMP assay and PCR for detection of ALV-J by agarose gel electrophoresis



Zhang, X. et al. 2010. J. Clin. Microbiol. 48(6):2116-2121

LAMP available for (among others):

FMD (Dukes et al. (2006): Arch Virol)

ND (Pham et al. (2005) J Clin Microbiol)

PRRS (Li et al. (2009) J Virol Methods)

ASF (Heather et al., (2010) J Virol Methods)

AIV H5 (Imai et al. (2006); Eiken, Loopamp®)

AIV H7 (Eiken, Loopamp®)

AIV H9 (Chen et al. (2008): J Virol Methods)

AIV M (Yoshida et al., 2011; J. Vet Med Science)

Influenza H1N1 (Kubo et al.(2010): J Clin Microbiol)

Evaluation of EIKEN Loopamp® (H5/H7)

J Vet Diagn Invest 22:61–66 (2010)

BRIEF RESEARCH REPORTS

Evaluation of two commercial loop-mediated isothermal amplification assays for detection of avian influenza H5 and H7 hemagglutinin genes

Alexander Postel, Tobias Letzel, Sieghard Frischmann, Christian Grund, Martin Beer, Timm Harder[1]

- **RT-H5/H7 LAMP (Eiken) was on average 1.000 fold less sensitive than conventional real-time RT-PCR**
- **Increasing of sensitivity caused decreasing of specificity**

Optimization of H5-LAMP

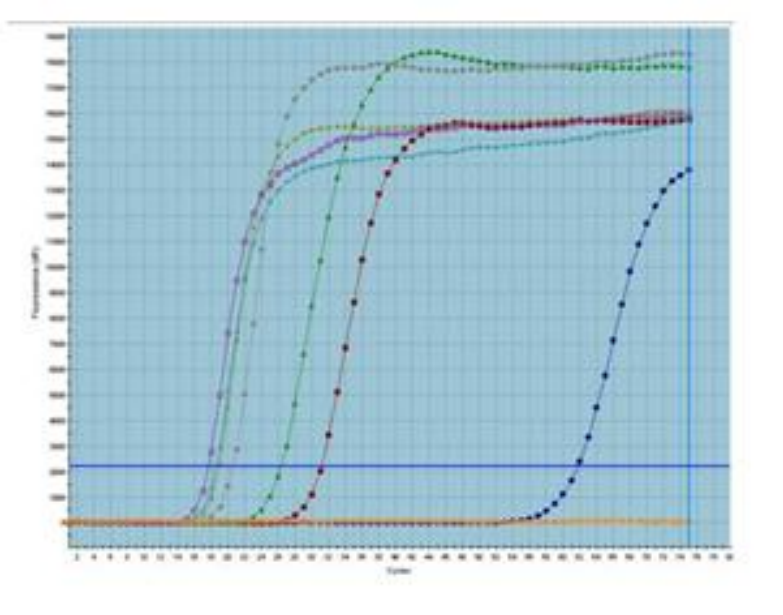
IAEA:

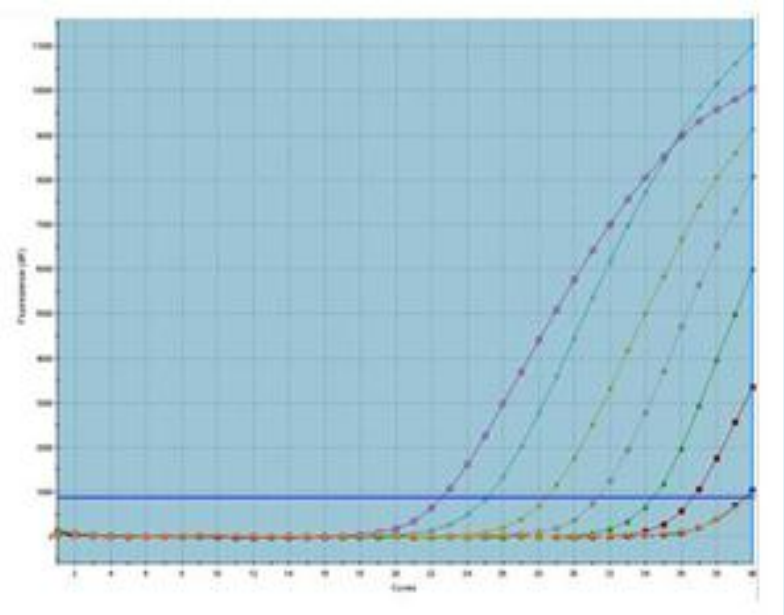
- Bsm DNA polymerase instead of Bst DNA polymerase
- Modified reaction mix
- Fluorescence dye EVA-Green™
- Lyophilized „all inclusive Master-mix tubes“
- Thermostable: 4°C for months
- Price (~3 US$)

Materials & Methods

- A panel of LP and HP H5 influenza viruses
- A panel of all other non-H5 subtypes
- RNA extraction using QIAamp Viral RNA kit (Qiagen) or rapid-short processing of samples
- A modified TaqMan one step RTqPCR to compare the results with LAMP.
- Animal experimet to collect fresh H5-positive diagnostic samples

Analytical Sensitivity of IAEA H5 kit

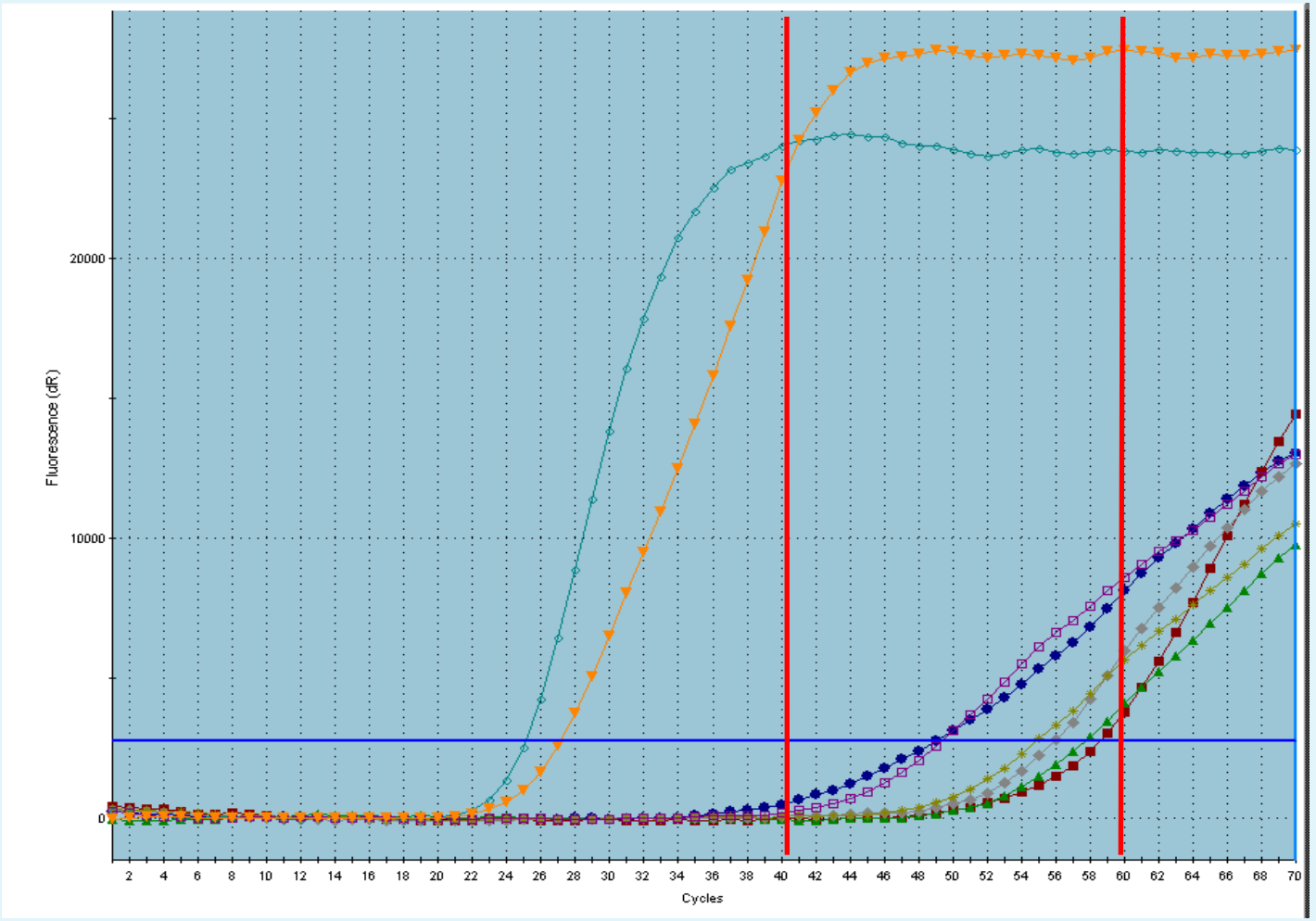




Well Name		min	Ct (NP)
original	10000	17.71	22.56
10 -1	1000	18.62	25.13
10 -2	100	18.95	28.45
10 -3	10	20.62	31.31
10 -4	1	26.37	34.45
10 -5	0.1	31.17	36.67
10 -6	0.01	61.79	39.55

Analytical Specificity of IAEA H5 kit

Our cut-off IAEA cut-off

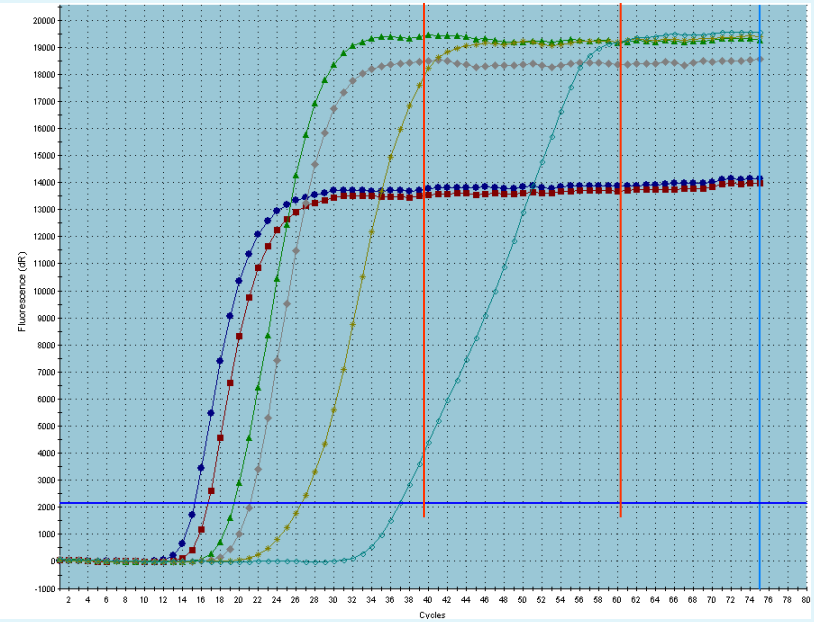


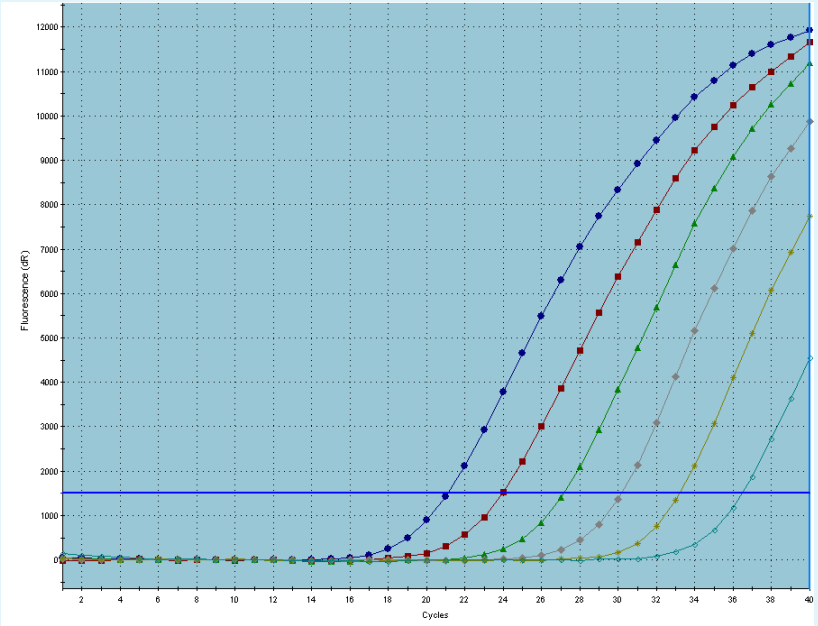
Positive samples

Negative samples & negative Controls

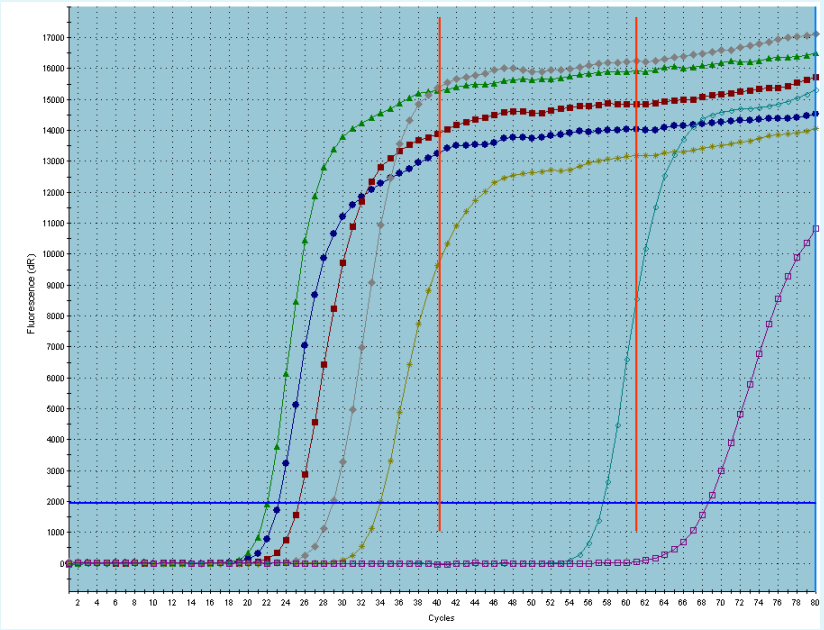
Isolate	subtype	LAMP		H5-rRT-PCR	Generic (NP) rRT-PCR
		40 min	60 min		
A/ egyptian goose/Germany/R668/10	H1 N1	neg	neg	neg	pos
A/swine/Germany/ R158/11	H1 N1	neg	pos	neg	pos
A/wild duck/Germany/R3111/07	H2 N9	neg	neg	neg	pos
A/whooper swan/R281/09	H2 N3	neg	pos	neg	pos
A/mallard/Iran/V10/04	H3 N8	neg	pos	neg	pos
A/mallard/Germay/R1102/10	H4 N6	neg	neg	neg	pos
A/ostrich/Germany/R48/10	H6 N2	neg	pos	neg	pos
A/mute swan/Germany/R2927/07	H6 N8	neg	neg	neg	pos
A/mallard/Iran/V31/04	H7 N3	neg	pos	neg	pos
A/anser spec./Germany/R44/2006	H8 N3	neg	neg	neg	pos
A/mallard/Iran/V16/04	H8 N4	neg	neg	neg	pos
A/chicken/Saudi-Arabia/R61/02	H9 N2	neg	neg	neg	pos
A/garganey/Iran/K8/03	H9 N2	neg	neg	neg	pos
A/eurasian coot/Germany/411/10	H10 N8	neg	neg	neg	pos
A/pigeon/Israel/543/2008/R713/09	H10 N7	neg	neg	neg	pos
A/dk/Kfar/Baruch Israel/2001_R715/09	H11 N2	neg	neg	neg	pos
A/duck/Alberta/60/76	H12 N5	neg	neg	neg	pos
A/black headed gull/Germany/R2622/06	H13N8	neg	neg	neg	pos
A/black headed gull/Atyrau/2915/08	H13N6	neg	pos	neg	pos
A/mallard/Gurjev/263/82	H14 N5	neg	neg	neg	pos
A/herring gull/Germany/R3309/07	H16N3	neg	pos	neg	pos
A/gadwall/Atyrau/2215/07	H16N3	neg	pos	neg	pos

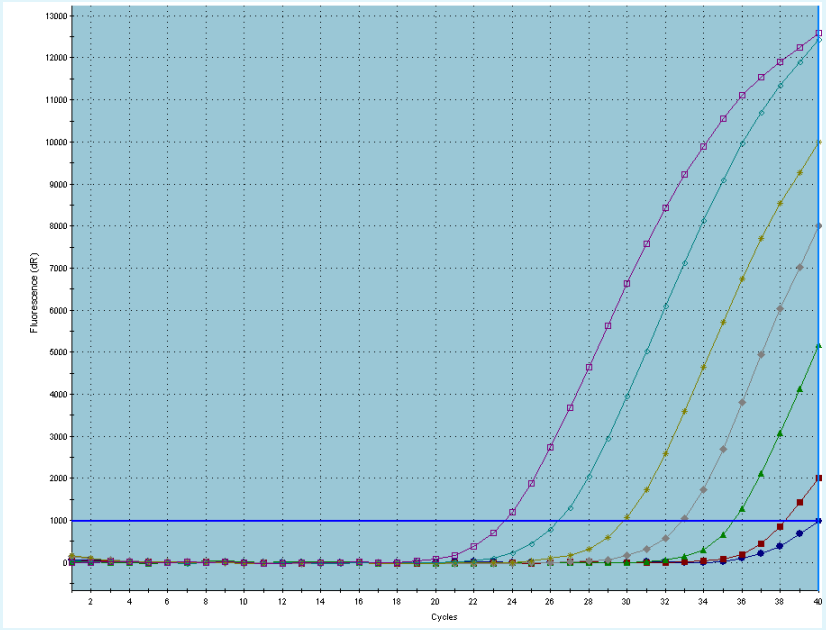
Analytical Sensitivity of IAEA H5 kit





Analytical Sensitivity of IAEA H5 kit





Analytical Sensitivity of IAEA H5 kit

H5 Isolate	H5-RT-LAMP (last positive dilution)		RT-qPCR (last positive dilution)	
	40 min	60 min	dilution	Cq
A/duck/Potsdam/1402-6/86 (H5N2)	10^{-6}	10^{-6}	10^{-6}	34.44
A/mallard/Germany/R2557/06 (H5N3)	10^{-6}	10^{-6}	10^{-6}	36.50
A/ostrich/Germany/R5-10/06 (H5N3)	10^{-4}	10^{-4}	10^{-6}	36.25
A/mallard/Germany/R409/05 (H5N3)	10^{-4}	10^{-4}	10^{-6}	35.50
A/Whooper swan/Germany/R535/09 (H5N2)	10^{-4}	10^{-5}	10^{-5}	36.18
A/teal/Germany/WV632/05 (H5N1)	10^{-4}	10^{-5}	10^{-5}	36.71
A/mallard/Germany/R771/08 (H5N3)	10^{-4}	10^{-5}	10^{-6}	37.64
A/Mallard/Alberta329/2006 (H5N9)	neg	10^{-4}	10^{-6}	37.15

Summary of results for IAEA LAMP:

	H5 LAMP	H5 Tetracore	H5 LFD	PanAI LFD
Sensitivity	++++	++++	+	++
Specificity (40 vs. 60)	++++/ +	++++	+	++++
Easy interpretation	++	++	++++	++++
Cool storage	+++	+++	++++	++++
Dependency to instruments	++	++	++++	++++
Fast results < 1 hour	++++	++	++++	++++
Cost	++++	+	++	+
Risk of cross-contmination	++	++	++++	++++

Advantages

- Quick results (within maximum 1,5 hours)
- High sensitivity (more sensitive than rapid test kits, LFD)
- No expensive equipment needed (*theoretically:* waterbath)
- Result can be read by naked eye (fluorescence, turbidity, colour change)

Disadvantages

- Borderline specificity
- High danger of lab contamination (need for good disposal system)

Acknowledgements

OIE:

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