

Evaluation of real-time PCR reagents for detection of influenza A virus RNA

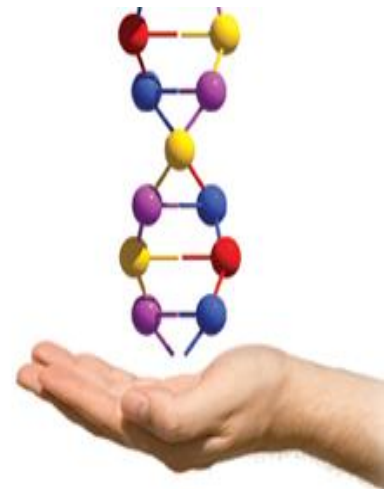
CAHLN 2017

June 7th 2017, Kristin Mesires, Ph.D.



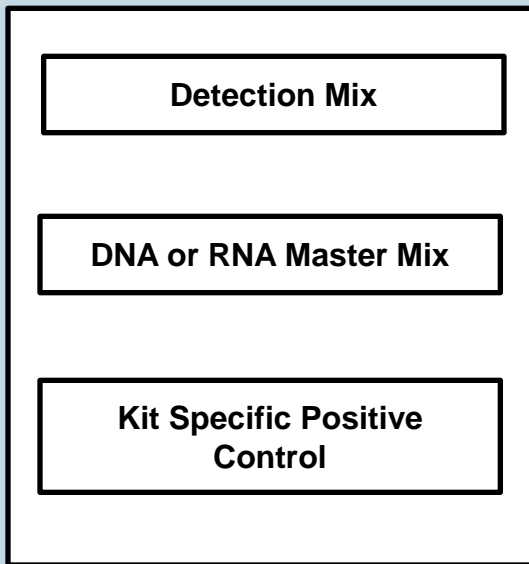
Agenda

- Overview of the RealPCR™ Modular Platform
 - Shared reagents
 - Standard real-time PCR cycling program
- Real-time PCR reagents for identification of Influenza A RNA
 - Analytical sensitivity
 - Inclusivity and Exclusivity
 - Clinical Sample Evaluation

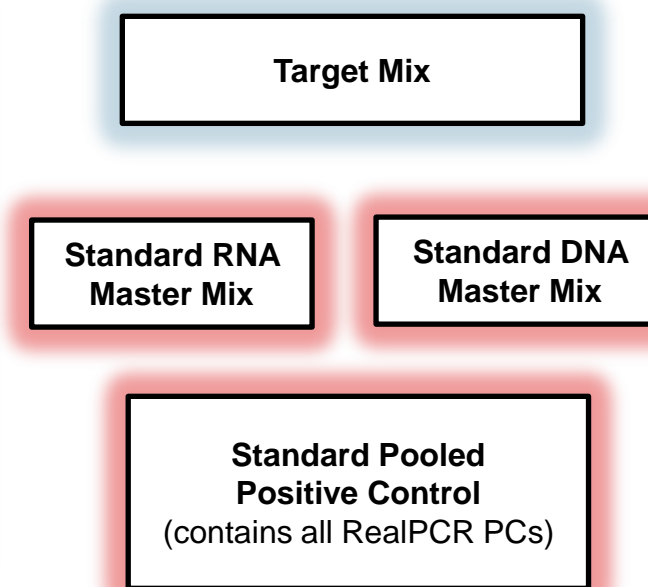


IDEXX RealPCR Platform

Kit Equivalent



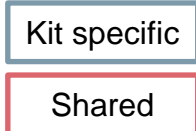
IDEXX RealPCR Platform



Target Mix (TMx) –
Target Primers & Probes
Internal Control (IC) primers & probes

Master Mix (MMx) –
Enzymes, nucleotides

Pooled Positive Control (PC) – Pool of target sequences for RealPCR reagents. Includes IDEXX Signature Sequence



Standard Real-Time PCR Program

- Standard cycling program for all RNA and DNA targets
- Multiple target testing in one real-time PCR run
- Minimizes reprogramming needs
- Designed to work on most common real-time PCR instruments

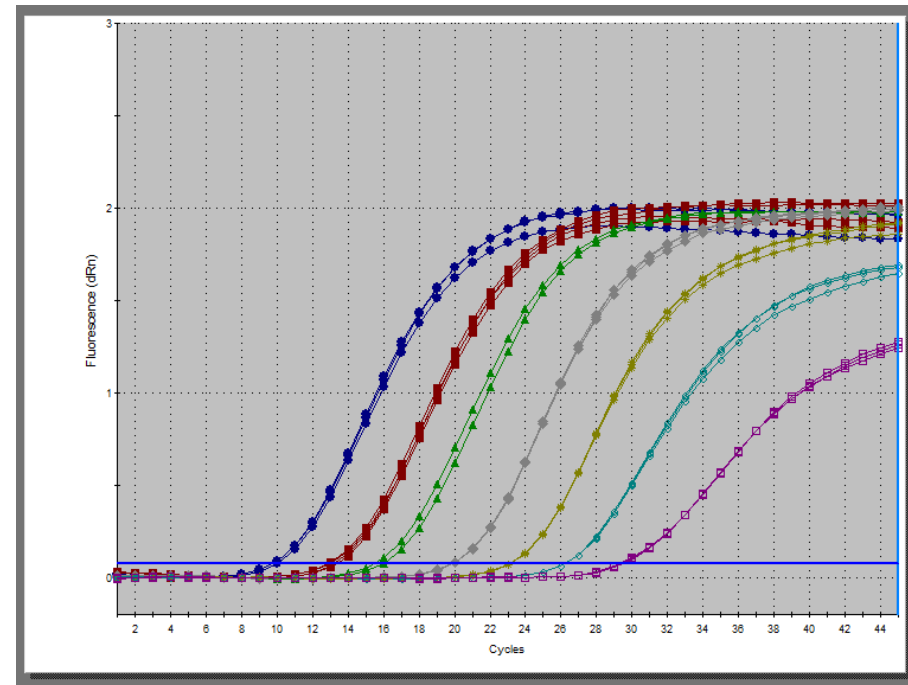
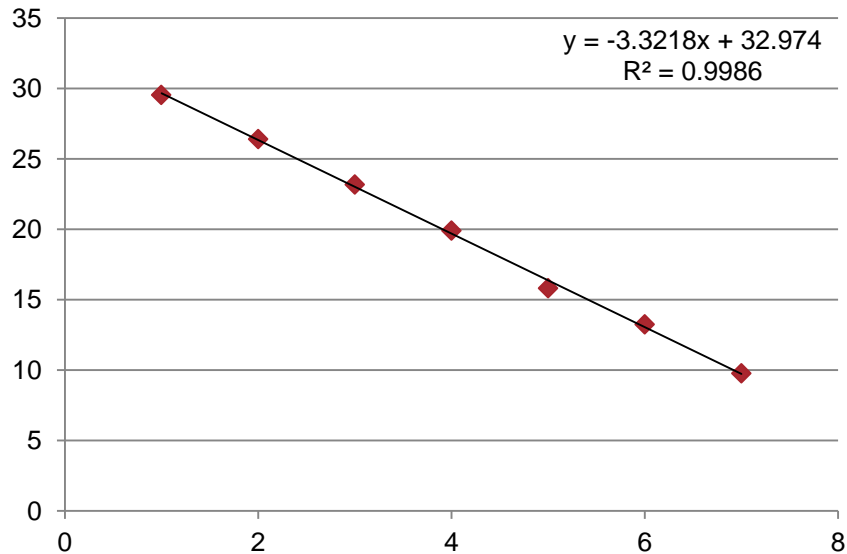
| Real-time PCR Step | Temperature | Time | Cycles |
|---|--------------|------------------|--------|
| Reverse transcriptase (not needed for DNA targets) | 50°C | 15 min | 1 |
| Denaturation | 95°C | 1 min | 1 |
| Amplification | 95°C 60°C | 15 sec 30 sec | 45 |

RealPCR Influenza A RNA Mix

- Detects Influenza A viral RNA
 - Amplifies target RNA using the FAM channel
- For screening, does not differentiate influenza subtypes
- Multispecies:
 - Samples from multiple host species have been evaluated (swine, avian, canine)
- Extraction:
 - Validated for use with bead and column extractions
- Control:
 - Utilizes an IPC to control for extraction efficiency (HEX/VIC channel)

Analytical Sensitivity

- 10-fold serial dilution series from 100 million to 10 copies per reaction of synthetic nucleic acid
- Detection of 3/3 reactions containing 10 copies
- Reactions contain 1,000 copies of RNA internal positive control (IPC)
- Efficiency of 100% over 7-log range

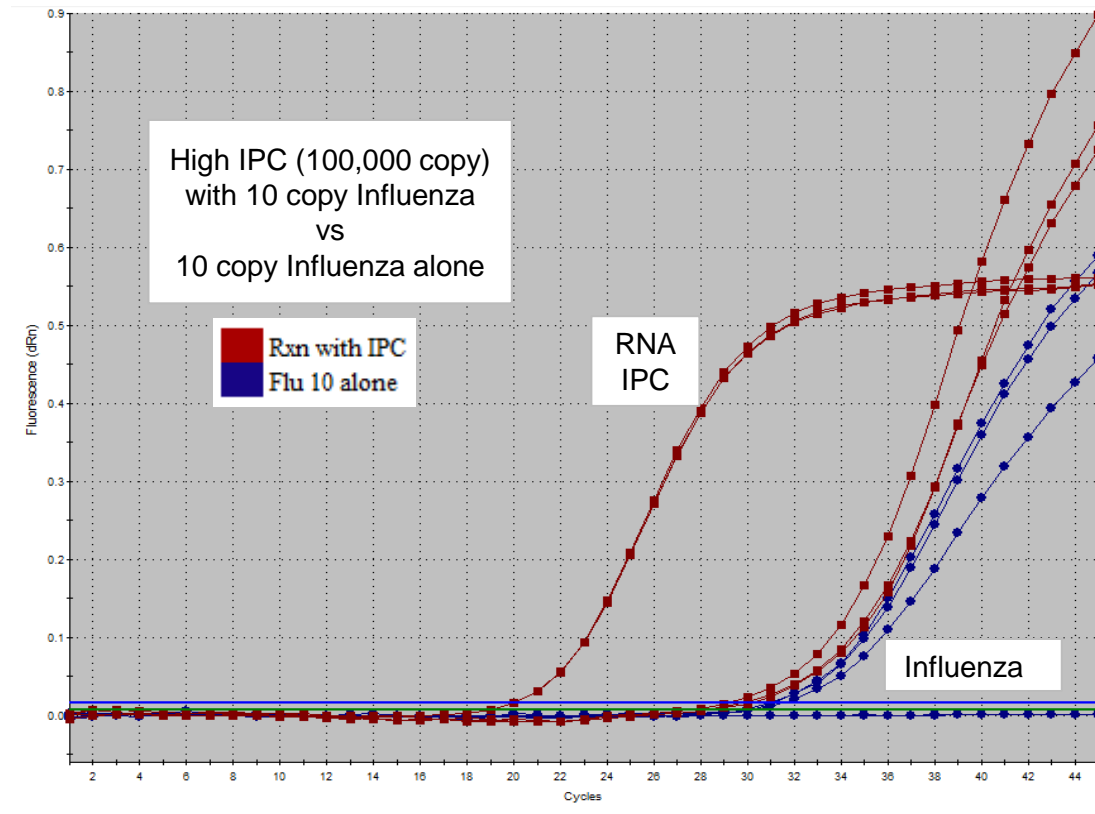


Test With Confidence™

IDEXX

Competition

- Positive samples have two reactions occurring simultaneously (competition)
 - One reaction amplifying the Influenza target, one amplifying the IPC
- Testing done to ensure the IPC reaction doesn't outcompete the Influenza A reaction



Inclusivity

❖ *In silico* and experimental analysis demonstrates reagents are inclusive of H and N subtypes.

- Experimental:
 - A panel of extracted RNA representing a variety of H and N subtypes was tested
 - H1-7; H10-11
 - N1-5; 7-9
 - All subtypes were detected

- *In silico*:
 - Detects all H and N subtypes except H17N10
 - H17N10 has 3 entries in database- all associated with bat

| RealPCR | | RealPCR | |
|---------|--------|---------|--------|
| Subtype | IAV Ct | Subtype | IAV Ct |
| H4N2 | 15.3 | H4N2 | 15.3 |
| H1N2 | 14.8 | H1N2 | 14.8 |
| H6N2 | 17.8 | H6N2 | 17.8 |
| H6N2 | 14.7 | H6N2 | 14.7 |
| H6N4 | 14.4 | H6N4 | 14.4 |
| H2N3 | 15.5 | H2N3 | 15.5 |
| H10N7 | 13.9 | H10N7 | 13.9 |
| H3N8 | 15.4 | H3N8 | 15.4 |
| H6N8 | 16.6 | H6N8 | 16.6 |
| H10N7 | 13.7 | H10N7 | 13.7 |
| H10N7 | 13.9 | H10N7 | 13.9 |
| H2N8 | 16.9 | H2N8 | 16.9 |
| H11N9 | 14.9 | H11N9 | 14.9 |
| H1N2 | 15.0 | H1N2 | 15.0 |
| H6N2 | 16.9 | H6N2 | 16.9 |
| H1N1 | 14.7 | H1N1 | 14.7 |
| H1N1 | 14.7 | H1N1 | 14.7 |

Exclusivity

❖ *In silico* and experimental analysis shows no cross-reactivity with other swine and avian respiratory pathogens.

- Swine Exclusivity List -
 - PRRSV, PCV, PRCV, Porcine Hemagglutinating Encephalomyolysis virus (HEV), porcine cytomegalovirus (PCMV), PRV, Nipah virus, Bordetella bronchiceptica, Pasteurella multocida, M. hyo, APP, Streptococcus suis, Haemophilus parasuis, Actinobacillus suis, Ascaris suis, Strongyloides ransomi
- Avian Exclusivity List-
 - Newcastle Disease Virus, Mycoplasma Gallisepticum, Mycoplasma synoviae, IBV, Avian Pneumovirus, ILT

Experimental exclusivity

| | RealPCR IAV RNA Ct |
|-------------|-----------------------|
| MG | -- |
| MS | -- |
| PM | -- |
| PCV2 | -- |
| PDCoV | -- |
| PRRS EU | -- |
| TGEV | -- |
| Mhyo | -- |
| Pos Control | 26.4 |

Clinical Sample Evaluation

- 328 samples
- Run on reference PCR and RealPCR Influenza A RNA Reagents
- Combination of IDEXX in-house evaluation plus field evaluation results
- Swine oral fluid, lung, and nasal swabs
- Avian tracheal swabs
- At least 18 different influenza subtypes

Clinical Sample Summary

❖ Good sensitivity and specificity across species, sample types, and test sites.

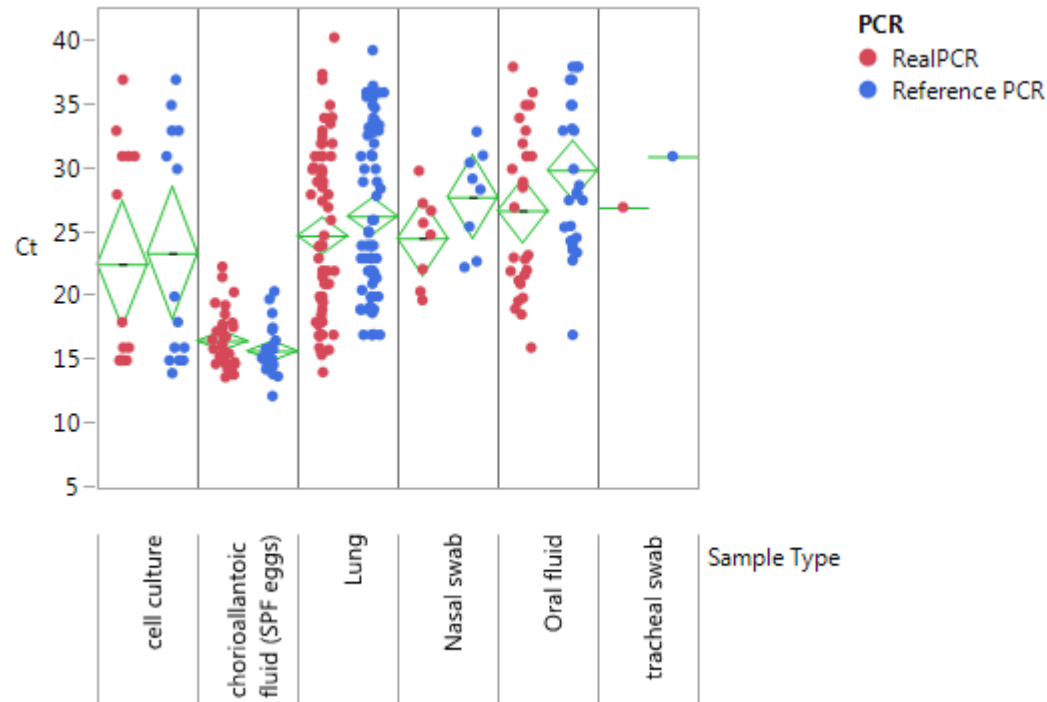
| | | Reference PCR | | Totals |
|--------------------|--------|---------------|-----|--------|
| | | Pos | Neg | |
| RealPCR IAV RNA | Pos | 174 | 1 | 175 |
| | Neg | 2 | 151 | 153 |
| | Totals | 176 | 152 | 328 |

| | |
|--------------------|-------|
| Sensitivity | 98.9% |
| Specificity | 99.3% |

| | Discrepant | |
|---|---------------------|---------------|
| | Reference PCR Ct | RealPCR Ct |
| 1 | 36.0 | - |
| 2 | - | 37.0 |
| 3 | 36.5 | - |

Clinical Sample Summary

❖ RealPCR Influenza A RNA Reagents generate earlier Cts than the reference PCR for most sample types



Summary

- IDEXX RealPCR™ Modular Platform
 - Shared reagents—use the same master mixes and positive control for all targets
 - Standardized and fast real-time PCR cycling protocol
- IDEXX RealPCR™ Influenza A RNA Mix
 - Work with the RealPCR Modular Platform
 - Good sensitivity and specificity on a broad range of sample types

Thank you!
Questions?

