



United States
Department of
Agriculture

Marketing and
Regulatory
Programs

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Service

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Final
Dec. 05. 2008

Assay Performance Characteristics Summary Sheet

Assay: Avian Influenza 2008 Pan-American (2008) H7 real-time reverse transcriptase - polymerase chain reaction (rRT-PCR) assay.

Disease: Avian Influenza (AI)

Type of Assay: rRT-PCR assay

Purpose of Assay: Screening (NAHLN), Confirmatory (NVSL reference laboratories)

Background Information:

The AI USDA/NVSL rRT-PCR 2008 H7 assay was developed as a collaborative effort between the Southeast Poultry Research Laboratory (SEPR), USDA, ARS and NVSL, APHIS. This assay is intended to be used to identify H7 specific RNA in clinical specimens and viral cultures. The H7 rRT-PCR assay targets a conserved region of the H7 hemagglutinin gene and has been shown to detect viral RNA from North American (NA) and South American H7 lineage viruses. The assay has been validated for detection of H7 in avian species and specimens. The assay will detect both highly pathogenic avian influenza (HPAI) and low pathogenicity avian influenza (LPAI) H7 viral RNA but will not differentiate HPAI from LPAI. Due to the diverse genetic coding of the H7 hemagglutinin gene, the 2008 H7 assay will detect LPAI H7 viruses found in NA wild birds as well as H7 viruses isolated from poultry in the U.S., Canada and Chile, but will not detect all Eurasian H7 lineage viruses.

The AI assay was initially validated to be performed on the Applied Biosystems 7500 Fast instrument with Ambion Ag-Path[®] RT-PCR chemistry and Ambion MagMAX[®] RNA extraction procedures. Subsequently, this assay has been shown to be equivalent when performed on the Applied Biosystems (AB) 7900 and Roche 480 real-time instruments with Ambion MagMAX[™] magnetic beads for the extraction of RNA. Equivalency studies have demonstrated a decrease in sensitivity with the Cepheid Smart Cycler instrument with Ambion Ag-Path[™] chemistry.

Sample Type: Tracheal/oropharyngeal (TR/OP) and cloacal (CL) swabs, tissue, and tissue pools

Species: Avian

Performance Characteristics:

Analytical sensitivity performance characteristics were determined using titrated H7 viruses. Analytical specificity studies were conducted with poultry and wild bird H7 viruses from the NVSL and SEPR reference collections. The rRT-PCR procedure was compared to virus isolation for the determination of the diagnostic sensitivity and specificity with experimentally exposed poultry swab specimens. Repeatability studies were conducted with titrated reference viruses.

Applied BioSystems 7500 (AB7500) Fast	
Analytical Sensitivity	10 ¹ EID ₅₀ per reaction

Total Samples Tested: n=1,578

ABI7500 Fast	Cutoff Ct Value ≤40
Diagnostic Sensitivity-avian	97.5%
Diagnostic Specificity-avian	82.4%

Platforms: AB7500, AB7900, Roche 480 and Cepheid Smart Cycler

Chemistries: Ambion Ag-Path[™]: AB7500 Fast, AB 7900, Roche 480
Qiagen One-Step[®]: Cepheid Smart Cycler

Performance Characteristics:

Diagnostic performance characteristics were determined using swab specimens collected from poultry and wild bird surveillance and swabs from experimentally inoculated chickens. Analytical performance characteristics were determined with reference AI viruses from the NVSL and SEPRL reference collections and AI viruses isolated from wild birds in 2006 and 2007.

Analytical Performance Characteristics:

Analytical Sensitivity: Analytical performance characteristics were determined by the NVSL and SEPRL using titrated H7 viruses obtained from the NVSL and SEPRL reference collections. Analytical sensitivity was determined by the SEPRL with in vitro-transcribed H7 HA gene RNA. Analytical performance characteristics have been shown to be reproducible.

AB7500 Fast: "Wet" Ambion Ag-Path® RT-PCR	
Analytical Sensitivity (titrated virus)	10 ¹ EID ₅₀ per reaction
Analytical Sensitivity (transcribed RNA)	10 ³ – 10 ⁴ copies or 100 fg ¹

Analytical Specificity: The 2008 Pan-American H7 assay was tested with RNA obtained from wild bird and poultry-origin influenza viruses representing 15 HA subtypes and isolates of poultry respiratory pathogens other than AI (avian paramyxovirus, infectious bronchitis virus, avian metapneumovirus, and infectious laryngotracheitis virus).

Isolate	Subtype	+/-	Isolate	Subtype	+/-	Isolate	Subtype	+/-
DK/MN/764-1489/81	H1N2 -		CK/CA/101247/01	H6N2 -		TY/CA/6889/80	H9N2 -	
A/NJ/8/76-Eq1 H1N7		-	Emu/TX/4259/93	H7N1 +		CK/Germany/N/48	H10N7	-
Mallard/ALB/77/77 H2N3		-	CK/NY/273874/03	H7N2 +		DK/England/56-Bel	H11N1	-
Waterfowl/GA/03 H2N9		-	TY/UT/24721-10/95	H7N3 +		Mallard/LALB/83	H12N5	-
DK/MN/LQWR604/79	H3N4 -		TY/OR/71	H7N3 +		Gull/MD/704/77	H13N6	-
Mallard/ALB/311/85 H3N6		-	CK/Chile/176822-1/02 H7N3		+	Mallard/Gurjev/263/82	H14N5 -	
DK/Czech/56 H4N6		-	CK/BC/314514/04	H7N3 +		Shearwater/Aust/1/73	H15N9	-
DK/England/62	H4N8 -		EQ/Prague/56	H7N7 -		Avian paramyxovirus	APMV	-
MSwan/MI/451072-2/06 H5N1		-	Mallard/AK/495762-38/06 H7N8		+	Infectious Bronchitis	Mass	-
DK/PA/454069/06	H5N1 -		TY/NE/505577/07	H7N9 +		Avian metapneumovirus	Subtype C	-
CK/WA/13413/84	H5N2 -		TY/Ontario/6118/67	H8N4 -		Infectious Laryngotracheitis virus	-	

In addition to the above viruses, 239 AI viruses isolated from North American wild birds were tested by the 2008 Pan-American H7 assay. Of the 239 viruses 82 H7 AI viruses of the H7N1, H7N3, H7N4, H7N7, and H7N8 subtypes were positive for H7 when tested by the AI 2008 Pan-American H7 assay. The remaining 157 non-H7 viruses (H1N1, H2N2, H2N3, H2N8, H3N1, H3N2, H3N5, H3N6, H3N8, H4N1, H4N2, H4N5, H4N6, H4N8, H5N1, H5N2, H5N3, H5N9, H6N1, H6N2, H6N8, H8N4, H10N3, H10N7, H11N2, H12N5) were negative for H7 by the 2008 Pan-American assay.

Applied Biosystems: AB7500

Performance Characteristics:

Analytical sensitivity performance characteristics were determined using ten-fold serial dilutions of H7N1 and H7N2 viral RNA. The standardized procedure (AVSOP1521) for AB software data interpretation was used for evaluation of all diagnostic and analytical data. Diagnostic and analytical validation was conducted with Ambion Ag-Path RT-PCR chemistry. The Qiagen One-Step® RT-PCR and Ambion Ag-Path™ chemistries have been validated for use with the AB7500 Fast with the AI matrix, H5 and 2002 H7 assays and have been shown to be equivalent. Optimization studies conducted with the 2008 H7 assay have shown a slight decrease in limit of detection (LOD) with the Qiagen One-Step® RT-PCR chemistry.

AB7500: Ambion Ag-Path™ “Wet” Chemistry	
Analytical Sensitivity	10 ¹ EID ₅₀ per reaction
AB7500: Qiagen One-Step® RT-PCR Wet” Chemistry	
Analytical Sensitivity	10 ² EID ₅₀ per reaction

Intra-assay Variation: Intra-run replicates of serially diluted H7 viral RNA over the linear range of the assay demonstrated minimal variation in cycle threshold values. A variation of < 2.0 cycle threshold (Ct) was demonstrated.

Applied Biosystems: AB 7900:

Performance Characteristics:

The assay was modified to accommodate instrument requirements for collection of fluorescence with a 3 step PCR. Modifications did not alter the diagnostic or analytical specificity of the assay. Analytical sensitivity performance characteristics were determined using ten-fold serial dilutions of H7N1 and H7N2 viral RNA. Limit of detection and analytical sensitivity were compared to data generated by the AB7500 instrument. A standardized procedure (AVSOP1523) for AB software data interpretation was developed based on AI matrix, H7, H5, APMV-1 matrix and vNDV analytical tests. The Qiagen One-Step® RT-PCR and Ambion Ag-Path™ chemistries have been validated for use with the AB7500 Fast with the AI matrix, H5 and 2002 H7 assays and have been shown to be equivalent. Optimization studies conducted with the 2008 H7 assay have shown a decrease in LOD with the Qiagen One-Step® RT-PCR chemistry.

AB7900: Ambion Ag-Path™ “Wet” Chemistry	
Analytical Sensitivity	10 ¹ EID ₅₀ per reaction
AB7900: Qiagen One-Step® RT-PCR “Wet” Chemistry	
Analytical Sensitivity	10 ²⁻³ EID ₅₀ per reaction

Intra-Assay and Inter-Assay Variation: Intra-assay and Inter-assay testing has demonstrated minimal variation and good reproducibility. A variation of < 2.0 Ct was demonstrated.

Roche: 480:

Performance Characteristics:

Analytical sensitivity performance characteristics were determined using ten-fold serial dilutions of H7N1 and H7N2 viral RNA. Limit of detection (LOD) and analytical sensitivity were compared to the AB7500. A standardized procedure for Roche software data interpretation has been developed based on AI matrix, H7, H5, APMV-1 matrix and vNDV analytical tests. Analytical studies conducted with the 2008 H7 assay have shown similar LOD with the Roche 480 and AB7500 Fast. Optimization studies conducted with the 2008 H7 assay have shown a slight decrease in LOD with the Qiagen One-Step® RT-PCR chemistry

Roche 480: Ambion Ag-Path™ “Wet” Chemistry	
Analytical Sensitivity	10 ¹ EID ₅₀ per reaction
Roche 480: Qiagen One-Step® RT-PCR “Wet” Chemistry	
Analytical Sensitivity	10 ¹⁻² EID ₅₀ per reaction

Intra-Assay and Inter-Assay Variation: Intra-assay and Inter-assay testing has demonstrated minimal variation and good reproducibility. A variation of < 2.0 Ct was demonstrated.

Cepheid: Smart Cyclers:

Performance Characteristics:

Analytical performance characteristics were determined using titered H7 AI viruses obtained from the NVSL reference collection. Analytical performance characteristics with the Ag-Path™ chemistry have shown a decrease in sensitivity with the Cepheid SmartCycler as compared to the AB 7500 Fast. Analytical performance has shown the Cepheid SmartCycler to be comparable to the AB 7500 Fast Ag-Path™ assay with Qiagen One-Step RT-PCR® chemistry and less sensitive than the AB7500 Fast Ag-Path™ with Ambion Ag-Path™ chemistry.

SmartCycler: “Wet” Ambion Ag-Path™ chemistry	
Analytical Sensitivity (tittered virus)	10 ^{4.5} EID ₅₀ per reaction
SmartCycler: “Wet Qiagen One-Step RT-PCR®	
Analytical Sensitivity (tittered virus)	10 ¹⁻² EID ₅₀ per reaction

Intra-Assay and Inter-Assay Variation: Intra-assay and Inter-assay testing has demonstrated approximately 1-2 logs difference in LOD with inter-assay replications. Inter-assay replications have demonstrated good reproducibility.

References:

1. Spackman, E., Ip, H.S., Suarez, D.L., Slemons, R.D., Stallknecht, D.E., Analytical Validation of a Real-time RT-PCR Test for Pan-American Lineage H7 Subtype Avian Influenza Viruses. Submitted to: J. Vet. Diagnostic Investigation. March 2008.
2. Suarez, D.L., Senne, D.A., Banks, J., Brown, I.H., Essen, S.C., Lee, C.W., Manvell, R. J., Mathieu-Benson, C., Moreno, V., Pedersen, J.C., Panigrahy, B., Rojas, H., Spackman, E., and Alexander, D.J. Recombination Resulting in Virulence Shift in Avian Influenza Outbreak. Emer. Inf. Dis. 10.4:693-699. 2004.

