DRG® Avian Influenza A Nucleoprotein Antigen Capture (EIA-4711)

Revised 28 March 2007 **RUO**

1 INTRODUCTION

Influenza viruses can be divided into three classes, A, B, and C, largely based upon conserved antigenic differences in the internal nucleoprotein. Influenza A virus, typically encountered more frequently than types B and C, and associated with the majority of serious epidemics, can be further subdivided into strains or subtypes based on antigenic differences in the external hemagglutinin proteins (H1-H16) and neuraminidase proteins (N1-N9).

A variety of wild waterfowl appear to be the predominant natural reservoir for Influenza A viruses and subtypes representing most of the hemagglutinin and neuraminidase combinations can be found circulating in these birds. Historically, human influenza virus infections have been associated with H1N1, H2N2, and H3N2 subtypes of influenza A, although a recent (1997) and significant outbreak in Hong Kong was identified as an H5N1 subtype. This outbreak was not only significant because it resulted in 18 human infections and 6 deaths, but it also represented the first known demonstration of avian influenza virus transmission to humans.

While influenza A subtype identification is extremely important (vaccine production, epidemiology), the rapid and accurate differentiation of influenza A from influenza B and C and other respiratory agents in humans and animals is also important (treatment and biosecurity).

DRG has developed a highly sensitive and specific enzyme immunoassay for the detection of Influenza A nucleoprotein antigen in complex sample matrices derived from both human and veterinary sources. The assay can be completed in less than 1.5 hr. and contains only one wash step. In addition, the test kit incorporates proprietary diluents that are designed to prevent the development of nonspecific signal derived from complex sample matrix effects and/or the nonspecific adsorption of reactive test components which result in improvements in both sensitivity and specificity .

The kit has been tested against a wide variety of influenza A subtypes for sensitivity and potentially interfering viruses and bacteria for specificity.

For Research Use Only

2 COMPONENTS

1.	Antigen Capture Plate (96 tests)	2 each
2.	Sample Preparation Reagent (1x)	12 ml
3.	Positive Control (1x)	1 ml
4.	Negative Control (1x)	2 ml
5.	Wash Buffer (20x)	30 ml
6.	Detection Antibody, HRP-labeled (1x)	22 ml
7.	Chromagen Solution (1x)	22 ml

8.	Stop Solution (1x)	22 ml
9.	Sample Dilution Tray	2 each

3 STORAGE

Store all kit components at 2-8°C.

Crystal formation may occur in the wash buffer concentrate during prolonged storage at 2-8° C. The crystals can be re-dissolved by swirling the bottle in warm tap water.

4 PROCEDURE

1. Remove the kit components from storage and allow to warm to room temperature.
2. Determine the number of test wells needed. Use one well for each sample.
   In addition, include one well for the **Positive Control** and three wells for the **Negative Control**.
3. To begin the assay, transfer 50 µl of **Sample Preparation Reagent** to the appropriate number of wells in the dilution tray provided.
4. Add 200 µl of each sample, positive control, and negative control to the Sample Preparation Reagent.
   Mix by pipetting up and down several times.
5. Transfer 100 µl of sample or control to the appropriate wells of the **Antigen Capture Plate**.
6. Cover the plate and incubate for 30 minutes at room temperature on a plate shaker set at moderate speed.
7. Add 100 µl of **1x Detection Antibody** to each well, cover the plate and incubate for 45 minuts on a plate shaker using the same settings (Step 6).
8. Wash the wells 6x with **1x Wash Buffer**.
9. Add 100 µl of **Chromagen Solution** to each well and incubate for 10 minutes on a plate shaker.
10. Stop the reaction by the addition of 100 µl of **Stop Solution**.
11. Shake the plate for 10-15 sec. to ensure that the reaction is uniformly stopped and then read the plate in a plate reader using a 450 nm filter.

5 QUALITY CONTROL

1. All negative control absorbance values should be ≤ 0.250.
2. The positive control absorbance value should be ≥ 0.500.
3. The calculated value for the positive control/cut-off should be ≥ 2 (see below).

6 DETERMINATION OF CUT-OFF AND INTERPRETATION OF RESULTS

1. To determine the **cut-off value**, calculate the mean of the three negative control absorbance values and multiply this value by 2.
2. To interpret the results for a given sample, divide the absorbance value for the sample by the cut-off value.

   Calculated sample values that are **> 1.1** are considered **reactive**.

   Calculated sample values that are **< 0.9** are considered **nonreactive**.

   Calculated sample values that are **≥ 0.9 and ≤ 1.1** are considered **equivocal**.

SYMBOLS USED WITH DRG ELISA´S

Symbol	English	Deutsch	Francais	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Distributeur	Distributeur	Distribuidor	Distributtore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση

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IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
Σ		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..

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