As of 20 May 2010 rm (Vers. 1.0) **USA: RUO**

Please use only the valid version of the package insert provided with the kit.

Influenza Ag A/B/H1N1 Dipstick
A rapid test for the qualitative detection of Influenza type A, type B and A(H1N1) antigen in human swab samples

Content
- 10 tests individually packed
- Buffer tube; assay diluent sufficient for 10 tests
- Test tubes, droppers, sterilized swabs
- 3 control swabs (A+, B+, negative)
- 1 package insert

For professional in vitro use only.

General Information

Method	sandwich type immunochromatographic assay
Shelf life	24 months from date of production
Storage	2-30°C
Sample	human swab samples
Results	after 10-15 minutes

Summary

The 2009 flu pandemic, or swine flu, is a global outbreak of a new strain of Influenza A virus subtype H1N1, a type of swine Influenza, that was first detected in people in the United States in April 2009. This virus continued to spread globally, On June 11, 2009, the WHO declared the outbreak a pandemic.
The Influenza Ag A/ B/ H1N1 Dipstick (RAP-5133) is a chromatographic immunoassay for the differential and qualitative detection of Influenza virus type A, type B and A(H1N1) antigens directly from nasal / throat / nasopharyngeal swab or nasal/nasopharyngeal aspirate specimens.
This assay is intended for professional use and for in vitro use only.
The Influenza Ag A/ B/ H1N1 Dipsticks are immobilized with mouse monoclonal anti-A(H1N1) hemagglutinin, anti-Influenza A and anti-Influenza B antibodies, respectively. And the specially selected antibodies are used as detector materials. These enable the this assay to identify of Influenza virus type A, type B and A(H1N1) antigens directly, with a high degree of accuracy.

Active ingredients of main components

- **1 test strip includes:**
  - Gold conjugate control (as main component): Anti-Chicken IgY-conjugated gold colloid (0.027±0.0054 µg)
  - Gold conjugate A(H1N1) (as main component): Mouse monoclonal anti-A(H1N1) hemagglutinin-conjugated gold colloid (0.081±0.0162 µg)
  - Gold conjugate A (as main component): Mouse monoclonal anti- Influenza A virus conjugated gold colloid (0.081±0.0162 µg)
  - Gold conjugate B (as main component): Mouse monoclonal anti-Influenza B virus conjugated gold colloid (0.081±0.0162 µg)
  - Test Line A(H1N1) (as main component): Mouse monoclonal anti-A(H1N1) hemagglutinin (1.068± 0.214 µg)
  - Test Line "A" (as main component): Mouse monoclonal anti-Influenza A virus (1.068±0.214 µg)
  - Test Line "B" (as main component): Mouse monoclonal anti-Influenza B virus (0.712±0.142 µg)
  - Control Line (as main component): Mouse monoclonal Anti-Chicken IgY(0.534 ± 0.107 µg)

- **Assay diluents:**
  - Tricine (0.4M), NaCl (q.s.), TritonX-100 (q.s.), Sodium azide (0.02%)

- **Control Swabs:**
  - Influenza A Positive Control swab: Inactivated human influenza virus A(H1N1) ($0.1x2^3$ HAU), Inactivated human influenza virus type A/H3N2 (0.1×2^3 HAU)
  - Influenza B Positive Control swab: Inactivated human influenza virus type B (0.1×2^5 HAU)
  - Influenza Negative Control swab: Formalin-inactivated *Streptococcus pyogenes* (5×10^5 Org.)

storage and stability

- The test strip should be stored at room temperature.
- The test strip is sensitive to humidity and as well as to heat. Perform the test immediately after removing the test strip from the foil pouch.
- Do not use it beyond the expiration. Do not freeze.
- Do not store at refrigerator.
- The shelf-life of the kit is as indicated on the outer package.
- This product is stable during 24 months from the date of manufacture.

specimen collection and storage

Sample collection

- Nasal swab specimens: To collect a nasal swab specimen, insert the sterile swab into the nostril that presents the most secretion under visual inspection. Using gentle rotation, push the swab until resistance is met at the level of the turbinate (less than one inch into the nostril). Rotate the swab a few times against the nasal wall.
- Throat swab specimens: To collect a throat swab specimen, vigorously rub a rayon throat swab on both tonsillar surfaces and the posterior pharynx. Remove swab from mouth and insert, tip down, into paper wrapper.
- Nasopharyngeal swab specimens: To collect a nasopharyngeal swab specimen, insert swab into nostril parallel to the palate and leave in place for a few seconds to absorb secretions.
- Nasal / Nasopharyngeal aspirate specimens:
  - Aspirate or wash volumes of 2 ~ 2.5 ml are recommended. Transfer the specimen into a clean, dry specimen container.
  - Excessive wash or aspirate volumes may result in decreased test sensitivity.
  - Process specimen as described in "Procedure of the test"

Specimen transport and storage

- Transport fresh specimens to the laboratory as rapidly as possible in a suitable liquid transport system maintained on ice or refrigerated at 2-8°C up to 3 days.
- Specimen should be tested as soon as possible after collection.
- If not use of transport media, specimens may be stored refrigerated (2-8°C), or at room temperature (15-30 °C), in a clean, dry, closed container for up to 8 hours prior to testing.
- If the specimen should be tested later, swab specimens should be placed into 1 ~ 2 ml of transport media or saline by direct extraction.

* Transport media: Use of the following transport media has been tested and found to be compatible with Influenza Ag A/ B/ H1N1 Dipstick (RAP-5133):
Saline, PBS, PBS+0.5%BSA, PBS+0.5%Gelatin, EMEM+1%BSA, EMEM+0.5%BSA, Trypticase soy Broth+0.5%BSA, Trypticase soy Broth+0.5% gelatin

Warnings and precautions

- For in vitro use only. DO NOT RE-USE test strip.
- The instruction must be followed exactly to get accurate results. Anyone performing an assay with this product must be trained in its use and must be experienced in laboratory procedures.
- Do not eat or smoke while handling specimens.
- Wear protective gloves while handling specimens. Wash hands thoroughly afterwards.
- Avoid splashing or aerosol formation.
- Clean up spills thoroughly using an appropriate disinfectant.
- Decontaminate and dispose of all specimens, reaction kits and potentially contaminated materials, as if they were infectious waste, in a biohazard container.
- Do not use the test kit if the pouch is damaged or the seal is broken.

- Do not mix and interchange different specimen.
- Components must not be used after the expiration date printed on the labels. Do not mix or interchange different batches/lots of reagents.
- Influenza Ag A/ B/ H1N1 Dipstick assay diluent contain a proprietary anti-microbial agent which presents no hazard to the user if normal laboratory safety precautions are followed.

TEST procecure

Specimen Extraction

All swab samples without transport media, should be extracted as following method.

- Allow test strip and extracted sample to room temperature prior to testing.
- Hold the disposable dropper vertically; draw up assay diluent in the bottle to the Fill Line as shown in the following test procedure figure (approximately 300µl). Transfer 300µl of assay diluents into a test tube.
- Insert the patient swab sample into the test tube. Swirl the swab head against the inside of the test tube at least five times while squeezing the tube. (see figure)
- Take the swab out while squeezing the tube and then dispose of the used swab in accordance with your biohazard waste disposal protocol.

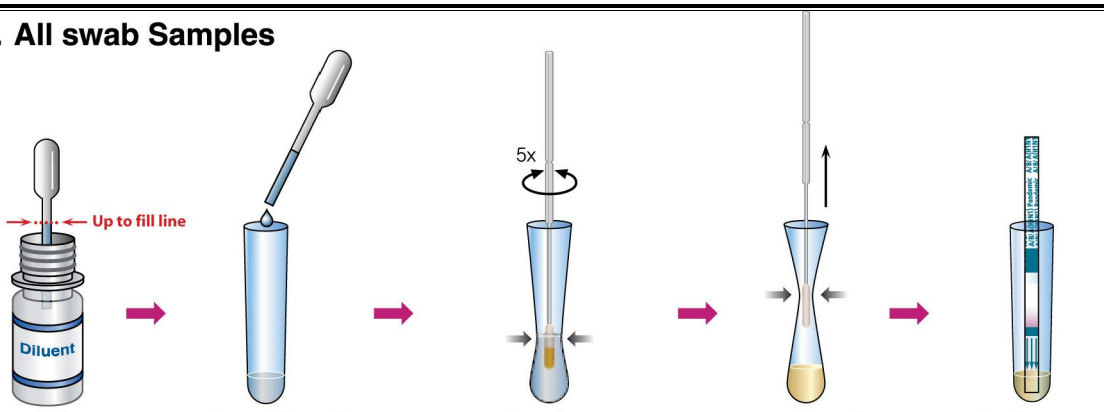
Nasal / Nasopharyngeal aspirate specimens and Swab samples to be placed into transport media by direct extraction

- Directly pipet both 100µl of extracted specimen, or aspirates and 100µl of assay diluent into a test tube. Mix specimen well.
- And then it should be followed according to Procedure of the test.

Reaction with Test Strip

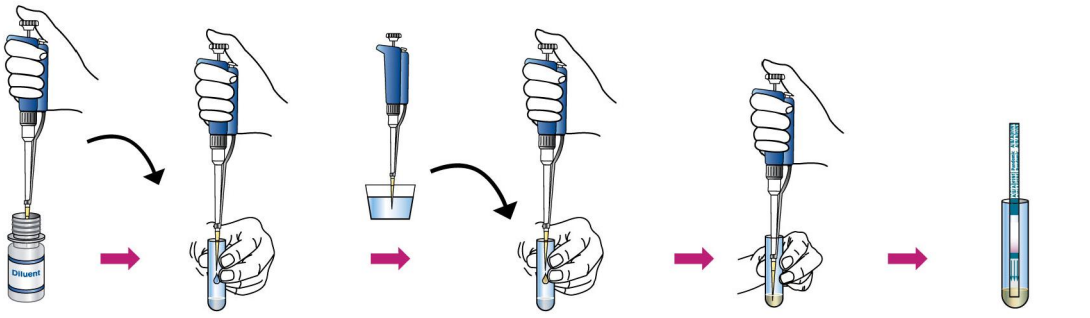
- Remove the test strip from the foil pouch prior to use.
- Place the test strip into the test tube with the arrows on the test strip pointing down. Do not handle or move the test strip until the test is complete and ready for reading.
- Read result at 10 ~ 15 minutes. Some positive results may appear soon.

I. All swab Samples



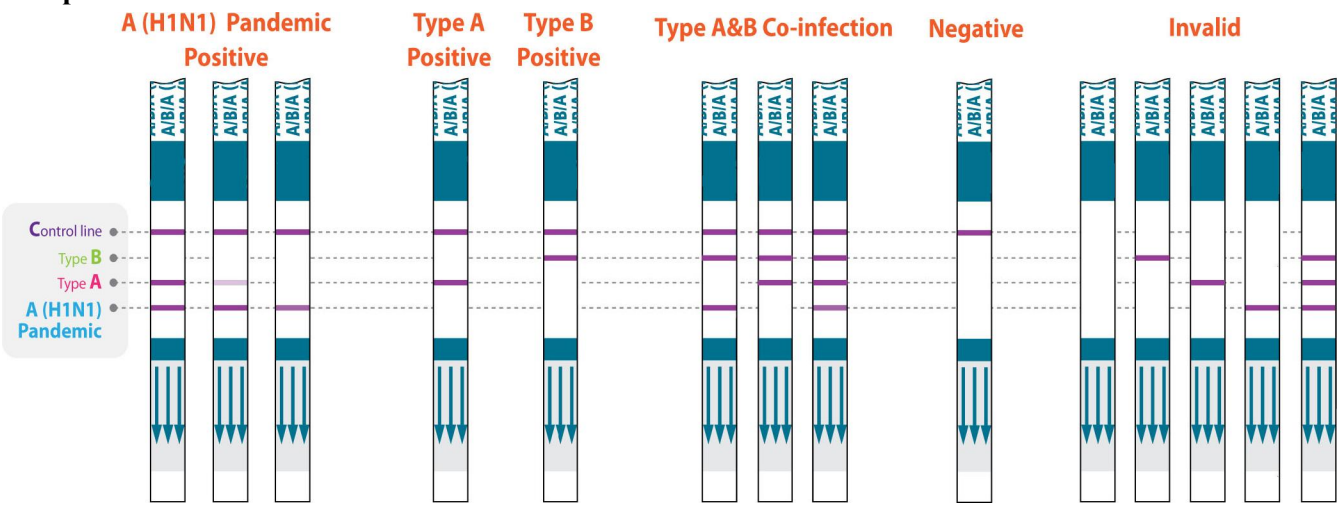
Up to Fill Line (approximately 300µl) | Transfer 300µl of assay diluent drawn from bottle to a test tube. | Insert the swab specimen, swirl the swab at least five times. | Remove the swab while squeezing. | Place the test strip into the test tube.

II. Nasal aspirate, nasopharyngeal aspirate specimen or Extracted specimen into transport media



Dispense 100µl of assay diluent into a test tube. | Directly pipet 100µl of extracted specimen, or aspirates, transfer specimen into the same test tube. | Mix well. | Place the test strip into the test tube.

interpretation of test results



Negative Result

- Only one control band appears.

Positive Result

- Positive for Influenza virus A (H1N1) [Two or three bands appear]: One purple color band ("A (H1N1)" line) or two purple color bands ("A (H1N1)" line & "A" line) and control band appear.
- Positive for Influenza virus type A [Two bands appear]: One purple color band ("A" line) and control band appear.
- Positive for Influenza virus type B [Two bands appear]: One purple color band ("B" line) and control band appear.
- Positive for Influenza virus type A&B (Co-infection suspected) [Three or four bands appear]: Two purple color band ("B" & "A" line or "B" & "A (H1N1)" line) or three purple color bands ("A", "B" and "A(H1N1)" line) and control band appear.

Invalid Result

- Control band fails to appear: If the purple color band is not visible within the result window after performing the test, the result is considered invalid. It is recommended that the specimen be re-tested using a new test kit.

limitations

- The Influenza Ag A/ B/ H1N1 Dipstick is to be used for the qualitative detection of influenza virus A(H1N1) antigens from nasal / throat / nasopharyngeal swab or nasal/nasopharyngeal aspirate specimens.
- Failure to follow the test procedure and interpretations of test results may adversely affect test performance and / or invalidate the test result.
- Test results must be evaluated in conjunction with other clinical data available to the physician.

- A negative test result may occur if the level of antigen in a sample is below the detection limit of the test or from improper sample collection. Negative test results are not intended to rule-out other non-influenza viral infections.

Quality control

Internal Quality control:

The test strip has Test Lines and Control Line on the surface of the test strip. All the Test Lines and Control Line in result window are not visible before applying any samples. The Control Line is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control line are working.

External quality control:

Control Procedures:

Positive and Negative control swabs should be tested according to the test procedure.

Specification

Influenza A Positive control swab should be interpreted as test line "A" & "A (H1N1)" Positive.
Influenza B Positive control swab should be interpreted as test line B positive.
Influenza Negative control swab should be interpreted as negative.

Performance characteristics

Sensitivity and Specificity [A(H1N1)]

Multi-center evaluation studies of the Influenza Ag A/ B/ H1N1 Dipstick (RAP-5133) shows as below;

Reference Assay: commercial RT-PCR	**Influenza Ag A/B/H1N1 Dipstick (RAP-5133)**		
	Positive	Negative	Total
(H1N1) Positive	199	60	259
Negative	0	78	78
(H1N1) Sensitivity	76,8% (199/259)		
Specificity	100% (78/78)		

In a comparison of the Influenza Ag A/ B/ H1N1 Dipstick (RAP-5133) versus a leading commercial RTPCR, results gave sensitivity of 76.8%(199/259), specificity of 100%(78/78). The Influenza Ag A/ B/ H1N1 Dipstick showed good correlation with commercial RT-PCR, total agreement was 82.2%.

Analytical specificity and cross reactivity

The Influenza Ag A/ B/ H1N1 Dipstick rapid test (RAP-5133) was evaluated with a total 9 bacterial and viral isolates. Bacterial and viral isolates were evaluated at a various concentration. These studies had been performed to demonstrate that there have been no cross reactivity test with 9 bacterial panels and virus panels on the Influenza Ag A/ B/ H1N1 Dipstick rapid test.

Analytical sensitivity:

Analytical sensitivity was established using a total of 17 human epidemic strains of influenza viruses:
16 influenza A and 1 influenza B

Precision

Within run precision

was determined by using 5 different replicates of 14 different specimens containing different type and concentrations of antigen. The negative and positive values were correctly identified 100% of the time.

Between run precision & batch-to-batch precision

was determined by using the 14 different specimens containing different type and concentrations of antigen in 3 different replicates with 3 different lots of test strips. Again negative and positive results were observed 100% of the time

Literature

1. Comparison of Lateral-Flow Immunoassay and Enzyme Immunoassay with Viral Culture for Rapid Detection of Influenza Virus in Nasal Wash Specimens from Children. Journal of Microbiology, Vol. 41, No. 5, May. 2003, p. 2132-2134
2. Comparison of Two Nested PCR, Cell Culture, and Antigen Detection for the Diagnosis of Upper Respiratory Tract Infections due to Influenza Viruses. Journal of Medical Virology 59 : 215-220(1999)
3. Simultaneous Detection and Typing of Influenza Viruses A and B by a Nested Reverse Transcription- PCR: Comparison to Virus Isolation and Antigen Detection by Immunofluorescence and Optical Immunoassay(FLU OIA). Journal of Microbiology, Vol. 39, No. 1, Jan. 2001, p.134-138

Symbols used with DRG Assays

Symbol	English	Deutsch	Français	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	Usage 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	Température 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	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità