ELISA kits available from ADI (see details at the web site)

Catalog#	Product Description
920-050-HAM	Human Anti-Influenza A virus (H1N1) IgM ELISA kit, 96 tests, Quantitative
920-060-HAA	Human Anti-Influenza A virus (H1N1) IgA ELISA kit, 96 tests, Quantitative
920-080-H5G	Human Anti-Influenza A Virus H5N1 IgG ELISA kit, 96 tests
920-085-H5M	Human Anti-Influenza A Virus H5N1 IgM ELISA kit, 96 tests
920-100-AIV	Chicken Anti-Avian Influenza A virus (AIV) IgG ELISA kit, 96 tests, Quantitative
920-105-AIM	Chicken Anti-Avian Influenza A virus (AIV) IgM ELISA kit, 96 tests, Quantitative
920-180-H9G	Chicken/Bird Flu/Influenza H9N1 Antibody ELISA kit
920-200-MHA	Human Anti-Influenza A virus M2 protein IgA ELISA kit (DIVA test), 96 tests, Quantitative
920-205-MHG	Human Anti-Influenza A virus M2 protein IgG ELISA kit (DIVA test), 96 tests, Quantitative
920-210-MHM	Human Anti-Influenza A virus M2 protein IgM ELISA kit (DIVA test), 96 tests, Quantitative
920-220-MMA	Mouse Anti-Influenza A virus M2 protein IgA ELISA kit (DIVA test), 96 tests, Quantitative
920-225-MMG	Mouse Anti-Influenza A virus M2 protein IgG ELISA kit (DIVA test), 96 tests, Quantitative
920-230-MMM	Mouse Anti-Influenza A virus M2 protein IgG ELISA kit (DIVA test), 96 tests, Quantitative
920-240-MRA	Rabbit Anti-Influenza A virus M2 protein IgA ELISA kit (DIVA test), 96 tests, Quantitative
920-245-MRG	Rabbit Anti-Influenza A virus M2 protein IgG ELISA kit (DIVA test), 96 tests, Quantitative
920-250-MRM	Rabbit Anti-Influenza A virus M2 protein IgG ELISA kit (DIVA test), 96 tests, Quantitative
920-300-H51	Chicken Anti-Avian Influenza virus (H5N1) IgG ELISA kit (1x96 wells)
920-320-MCG	Chicken Anti-Influenza A virus M2 protein IgG ELISA kit (DIVA test), Quantitative
920-325-MCM	Chicken Anti-Influenza A virus M2 protein IgM ELISA kit (DIVA test), Quantitative
920-330-MSG	Swine/Pig Anti-Influenza A virus M2 protein IgG ELISA kit (DIVA test), Quantitative
920-335-MSM	Swine/Pig Anti-Influenza A virus M2 protein IgM ELISA kit (DIVA test), Quantitative
920-360-HNG	Human Anti-Influenza A virus Nucleoprotein (NP/H1N1) IgG ELISA kit, Quantitative
920-365-HNM	Human Anti-Influenza A virus Nucleoprotein (NP/H1N1) IgM ELISA kit, Quantitative
920-370-H73	Chicken Anti-Avian Influenza virus HA IgG (H7N3) ELISA kit, 96 tests, Quantitative
920-380-MKA	Monkey Anti-Influenza A virus M2 protein IgA ELISA kit (DIVA test), Quantitative
920-385-MKG	Monkey Anti-Influenza A virus M2 protein IgG ELISA kit (DIVA test), Quantitative
920-390-MKM	Monkey Anti-Influenza A virus M2 protein IgM ELISA kit (DIVA test), Quantitative
920-405-HBM	Human Anti-Influenza B virus IgM ELISA kit, 96 tests, Quantitative
920-410-HBA	Human Anti-Influenza B virus IgA ELISA kit, 96 tests, Quantitative
980-100-PHG	Human Anti-H. Influenzae B (Hib) polyribosyl phosphate (PRP) IgG ELISA Kit,
980-110-PHM	Human Anti-H. Influenzae B (Hib) polyribosyl phosphate (PRP) IgM ELISA Kit,
980-120-PMG	Mouse Anti-H. Influenzae B (Hib) polyribosyl phosphate (PRP) IgG ELISA Kit
980-130-PRG	Rabbit Anti-H. Influenzae B (Hib) polyribosyl phosphate (PRP) IgG ELISA Kit,
980-140-PRM	Rabbit Anti-H. Influenzae B (Hib) polyribosyl phosphate (PRP) IgM ELISA Kit,
980-150-PKG	Monkey Anti-H. Influenzae B (Hib) polyribosyl phosphate (PRP) IgG ELISA Kit,
980-200-PDG	Human Anti-H. Influenzae B (Hib) protein D (PDG) IgG ELISA Kit, 96 tests
980-200-PDM	Human Anti-H. Influenzae B (Hib) protein D (PDG) IgM ELISA Kit, 96 tests
980-220-PDG	Mouse Anti-H. Influenzae B (Hib) protein D (PDG) IgG ELISA Kit, 96 tests
980-230-PDG	Rabbit Anti-H. Influenzae B (Hib) protein D (PDG) IgG ELISA Kit, 96 tests
980-250-PDG	Monkey Anti-H. Influenzae B (Hib) protein D (PDG) IgG ELISA Kit, 96 tests
980-HIB-AG1	Human Anti-H. Influenzae B (Hib) polyribosyl phosphate (PRP) antigen (vaccine) ELISA Kit, 96 tests
980-VID-Hib-48	ID-VAC H. Influenzae B (Hib-PRP) vaccine Identification ELISA Kit (Confirm the presence of active ingredients in commercial vaccines), 48 tests
980-VID-Hib-96	ID-VAC H. Influenzae B (Hib-PRP) vaccine Identification ELISA Kit (Confirm the presence of active ingredients in commercial vaccines), 96 tests
AE-300600-1	Mouse Sendai/(SeV/Parainfluenza virus 1) IgG ELISA Kit, 96 tests
AE-300610-1	Rat Sendai/(SeV/Parainfluenza virus 1) IgG ELISA Kit, 96 tests
AE-300620-1	Guinea Pig Sendai/(SeV/Parainfluenza virus 1) IgG ELISA Kit, 96 tests

Instruction Manual No. M-920-050-HAM

Human Anti-Influenza A IgM ELISA KIT
Cat. # 920-050-HAM, 96 tests

For Detecting Human IgM antibodies against Influenza A (Swine flu) in Serum or Plasma

For In Vitro Research Use Only (RUO)

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

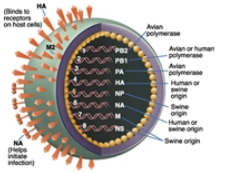
Human Influenza A IgM ELISA KIT Cat. # 920-050-HAM (96 tests)

Kit Components (96 tests)	Cat #
Influenza A IgM antigen coated strip plate, (8x12 strip or 96 wells) # 920051	1 plate
Influenza A IgM **Calibrator A, (1 U/ml) -ve Control** #920052A; 2 ml	1 vial
Influenza A IgM **Calibrator B, (10 U/ml) Cut-off Standard** #920052B, 2 ml	1 vial
Influenza A IgM **Calibrator C, (40 U/ml) Weak +ve control** #920052C, 2 ml	1 vial
Influenza A IgM **Calibrator D, (150 U/ml) +ve Control** #920052D, 2 ml	1 vial
controls contain 0.02 % methylisothiazolone and 0.02 % bromonitrodioxane as preservative (or see lot sp. conc on the vial)	
Anti-Human IgM-**HRP Conjugate**, (15 ml) #920053	1 bottle
Sample Diluent, 60 ml #920050SD	1 bottle
Wash buffer (10X) 60 ml # 920050WB	1 bottle
TMB Substrate Solution, 15 ml #920050TM	1 bottle
Stop Solution, 15 ml # 920050ST	1 bottle
Plate covers for plates and bags for storing un-used antigen strips	1 bottle
Complete Instruction Manual, M-920-050-HAM	1

Intended Use

ADI **Influenza A IgM** Antibody ELISA Test Kit is an indirect ELISA for the qualitative (-ve or +ve) or quantitative detection or measurement of IgM class of antibodies against two most common strains of swine flu **(H1N1+H3N2)** antigens in human serum and plasma. This kit is for research use only (RUO) and not for diagnosis, cure or prevention of the disease.

Introduction



Swine influenza or swine flu, also called pig influenza, hog flu and pig flu, is an infection caused by any one of several types of swine influenza viruses. Swine influenza virus (SIV) or swine-origin influenza virus (S-OIV) is any strain of the influenza family of viruses that is endemic in pigs. The known SIV strains include influenza C and the subtypes of influenza A known as H1N1, H1N2, H2N1, H3N1, H3N2, and H2N3. Swine influenza virus is common throughout pig populations worldwide. Transmission of the virus from pigs to humans lead to human flu or Zoonotic swine flu. People with regular exposure to pigs are at increased risk of swine flu infection. Vaccination of these workers against influenza and surveillance for new influenza strains among this population may therefore be an important public health measure.

According to the Centers for Disease Control and Prevention (CDC), in humans the symptoms of the 2009 "swine flu" H1N1 virus are similar to those of influenza and of influenza-like illness in general. Symptoms include fever, cough, sore throat, body aches, headache, chills and fatigue. The 2009 outbreak has shown an increased percentage of patients reporting diarrhea and vomiting. The 2009 H1N1 virus is not zoonotic swine flu, as it is not transmitted from pigs to humans, but from person to person. The most common cause of death is respiratory failure. Other causes of death are pneumonia (leading to sepsis), high fever (leading to neurological problems), dehydration (from excessive vomiting and diarrhea), and electrolyte imbalance and kidney failure. Fatalities are more likely in young children and the elderly.

Influenza spreads between humans when infected people cough or sneeze, then other people breathe in the virus or touch something with the virus on it and then touch their own face. Swine flu cannot be spread by pork products, since the virus is not transmitted through food. The swine flu in humans is most

Quality Control

The test results are only valid if the test has been performed following the instructions. All standards and kit controls must be found within the acceptable ranges as stated on the vials. The positive control must show at least double the OD of the cut-off standard. If criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. In case of any deviation the following technical issues should be proven (reagents, protocol, equipments, etc).

PERFORMANCE CHARACTERISTICS

Intra-Assay-Precision 8.3 %

Inter-Assay-Precision 10.8%

Inter-Lot-Precision 1.3- 9.9%

Analytical Sensitivity 1.17 U/mL

Clinical Sensitivity 100 %

Clinical Specificity 100%

Recovery 83- 90 %

Linearity 73- 122 %

Interferences

No interferences to bilirubin up to 0.3 mg/mL; Hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL.

Cross Reactivity:

No cross reactivity to RSV, adenovirus, and parinfluenza.

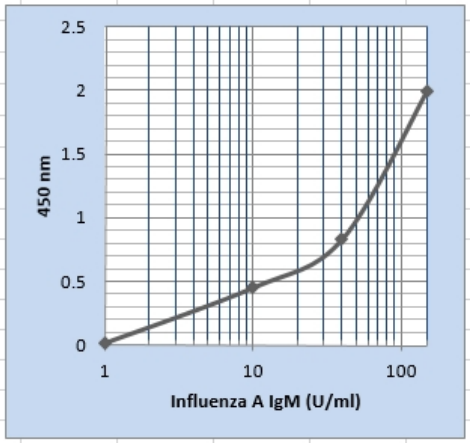
References:

Drescher J (1994) J. Virol. Methods. 47, 307-319; Marcante R (1996) New Microbiolo. 19, 141-147; Moldoveanu, Z et al (1995) Vaccine 13, 1006-1012; Naikhin, An et al (1997) Vopr. Virusol. **42**(5): 212-6; Shafer Al et al (1998) Avian Dis. 42, 28-34

WORKSHEET OF A TYPICAL ASSAY

Wells	Stds/samples	Mean A450	Results
A1, A2	**Calibrator A** **Negative Control (0 U/ml)**	0.011	
B1, B2	**Calibrator B** **Cut-off standard (10 U/ml)**	0.446	
C1, C2	**Calibrator C** **Weak Positive (40 U/ml)**	0.826	
D1, D2	**Calibrator D** **Positive Control (150 U/ml)**	1.994	
E1, E2	Sample 1	0.40	Negative
F1, F2	Sample 1	1.50	Positive

NOTE: These data are for **demonstration purpose only**. Use the values that are generated with each test.



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Calculation of Results

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 parameter logistics or Logit-Log.

For the calculation of the standard curve apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used). The concentration of the samples can be read from the standards curve.

The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor. Samples showing concentrations above the highest standard have to be diluted as described in Test Procedure and reassayed.

contagious during the first five days of the illness, although some people, most commonly children, can remain contagious for up to ten days. Diagnosis can be made by sending a specimen, collected during the first five days, for analysis.

The 2009 flu pandemic vaccines are the set of influenza vaccines that have been developed to protect against the pandemic H1N1/09 virus. Two types of influenza vaccines are available: TIV (flu shot (injection) of trivalent (three strains; usually A/H1N1, A/H3N2, and B) inactivated (killed) vaccine) or LAIV (nasal spray (mist) of live attenuated influenza vaccine.). TIV works by putting into the bloodstream those parts of three strains of flu virus that the body uses to create antibodies; while LAIV works by inoculating the body with those same three strains, but in a modified form that cannot cause illness. LAIV is not recommended for individuals under age 2 or over age 49, but might be comparatively more effective among children over age two. Both these types of vaccine are usually produced by growing the virus in chicken eggs.

ADI Human influenza A IgM measures antibody is serum or plasma samples. We also have kits to anti-influenza IgG and IgM in human, human, rabbits, and chickens.

PRINCIPLE OF THE TEST

Alpha Diagnostic's Influenza A IgM antibody test kit is based on the principle of the enzyme immunoassay (EIA). Influenza A antigens are bound on the surface of the microtiter strips. Diluted patient serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgM antibodies of the serum and the immobilized Influenza A takes place. After an incubation step, the plate is rinsed with wash solution, in order to remove unbound material. Bound antibodies are detected with anti-human IgM-HRP conjugate. After a further washing step, the substrate (TMB) solution is added. The color (blue) development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The color is measured using an ELISA reader at 450 nm. The concentration of the IgM antibodies is directly proportional to the intensity of the color. Results are obtained by comparing the A450 of the samples with the supplied negative and positive calibrators.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5µl, 100µl, 500µl) and multichannel pipet with disposable plastic tips. Bidistilled water, reagent troughs, Orbital shaker, plate washer (recommended) and ELISA plate Reader (450nm).

PRECAUTIONS

Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed. All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless, precautions like the use of latex gloves have to be taken. Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly. All reagents have to be brought to room temperature (18 to 25 °C) before performing the test. Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided. It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions. When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time. In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used. No reagents from different kit lots have to be used, they should not be mixed among one another. All reagents have to be used within the expiry period. In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation. The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.
TMB (substrate), Diluted H2SO4 (1N, stop solution), and Thimerosal (0.02% v/v in standards, conjugate diluent and HRP-conjugates).

SPECIMEN COLLECTION AND HANDLING

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 48 hours, for a longer storage they should be kept at -20 °C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results. For the performance of the test the samples (not the standards) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).

REAGENTS PREPARATION

Dilute Wash buffer 1:10 with water. Store diluted buffer at 4oC for 1 month. (If during the cold storage crystals precipitate, the concentrate should be warmed up at 37 degrees C for 15 minutes.

All reagents must be at room temperature prior to their use.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{o}C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. The unused portions of the standards should be stored at 2-8^{o}C or stored frozen in small aliquots and should be stable for 3 months.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. **All samples should be diluted 1:101 (5 ul samples in 500 ul sample diluent)**. It is recommended to prepare a parallel replica plates containing all sample for quick transfer to the coated plate. Label or mark the microtiter well strips to be used on the plate. Dilute the wash buffer with water (1:10),

1. Dispense 100 ul diluent in 1 well to be used as blank. Pipet **100 ul of** Prediluted controls, and samples (diluted 1:101) into appropriate wells in *duplicate*. See worksheet of a typical set-up on page 5. Cover the plate, mix gently for 5-seconds and incubate at room temp for **60 min**.

2. Aspirate the well contents and blot the plate on absorbent paper. Immediately, **wash the wells 3 times** with 250-300 ul of 1X wash buffer. We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

3. Add **100 ul anti-IgM-HRP conjugate** to all wells leaving one empty for the substrate blank. Mix gently for 5-10 seconds. Cover the plate and incubate for **30 minutes** at room temp (25-28oC).

4. Wash the wells 4 times as in step 3.

5. Add **100 ul TMB substrate solution**. Mix gently for 5-10 seconds. Cover the plate and incubate for **20 minutes** at room temp. Blue color develops in positive controls and samples.

6. Stop the reaction by adding **100 ul of stop solution** to all wells. Mix gently for 5-10 seconds to have uniform color distribution (blue color turns yellow).

7. Measure the **absorbance at 450 nm** using an ELISA reader within 60 min.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4^{o}C. Do not touch the bottom of the wells.

Interpretation of Results

The mean values for the measured absorptions are calculated after subtraction of the blank values from the controls and standards.

Negative	<8 U/ml
Equivocal	8-12 U/ml
Positive	>12 U/ml

The results themselves should not be the only reason for any therapeutically consequences. They have to be correlated to other clinical observations and diagnostic tests.

Expected values

Isotype	n	Interpretation		
		Positive	Equivocal	Negative
Infl. A IgM	72	2.8%	0.0%	97.2%