using an eliza to test for avian flu essay sample

This experiment was performed in a plastic microtiter plate. Wells 1-12 of rows A and B were filled with 40 µL of stimulated antigen by using a micropipette. A clean pipette tip was used every time a new sample or reagent was added to the wells. Each sample was tested in triplicate and the amount of sample or reagent used was always 40 µL. The positive control were added to the first three wells of row A (A1, A2, A3), then the same amount of negative control was added to the next three wells (A4, A5, A6). Similarly, samples for Patient A, B, C, D, E, and F were added to wells A7-B12 as seen in Figure 1.

Figure 1: Sample microtiter plate used for this experiment. Legend: +C=> positive control, -C=> negative control, A-F => patient samples A through F. The stimulated secondary antibody was added to all the wells in rows A and B. Lastly, 40 µL of stimulated chromogen was added to all 24 wells (A1-12, B1-12). The plate was incubated at room temperature for 10 minutes. Observations were recorded. Results

Figure 2: Microtiter plated after a 10 min incubation period at room temperature. The positive signs indicate the presence of the color purple in the wells after incubation. The intensity is displayed by the number of + sings present in each well. The highest intensity was +++ for the positive control (A1-A3). Negative signs indicate the lack of the color purple in the wells. The negative control was used for comparison (A4-A6). Patient A, D, and F showed signs of infection by avian flu in different degrees. Table 1: Summary of results from the Elisa test for Patients A-F . Patients were tested for Avian Flu (H5N1). The presence of an infection was indicated by the intensity of the color purple observed in the microtiter plate. Patient

Patient Background

Results

Interpretation

Comments

+ Control

Control

+++

Intense purple color

Results as expected

– Control

Control

–

No purple color

Results as expected

A

Sick chicken in central Aisa

++

Purple color

observed. Less intense then in positive control

Patient is infected

B

Employee of Patient A

–

No purple color observed

Patient is not infected

C

Wife of Patient B

–

No purple color observed

Patient is not infected

D

Tender of chickens in central Europe. Owner of chickens that are named as

Patient E and F +

Purple color observed. Less intense then both the control and Patient A

Patient is infected. Infection is less acute.

E

Chicken from Patient’s D flock

–

No purple color observed

Patient is not infected

F

Chicken from Patient’s D flock

++

Purple color observed of the same intensity as Patient A

Patient is infected

Discussion

The results of our experiment determined that Patient A, Patient D, and Patient F were infected with the H1N5 virus. Patient A and F showed a more acute infection than Patient D. This was determined by the more intense color seen in the Eliza test, which indicated that more of the enzyme attached to the secondary antibody was present. This suggested higher

levels of secondary and thus primary antibody, indicating that the virus has higher affinity for the chicken cells compared to human cells. Patients B, C and E showed no sign of infection. There is a possibility that they have caught the virus, but the body has not been able to build an immune response against it yet. In this case the Eliza test is not a good indicator of the presence of the virus, since no antibodies have been produced by the patients. By the results it is possible to deduce people who have been in close contact with sick chickens were at a higher risk of infection. Patient B and D have both been in close contact with sickly chickens.

However, it is possible that patient B is more careful when handling chickens because of his line of work, compared to Patient D. Since Patient D is tending for his chickens at home he might not be as aware of the safety measures that need to be taken when he notices sick chickens in his flock. It is very likely that patient D got infected when caring for sick chickens, or maybe when eating undercooked poultry products. The presence of the virus in one of Patient’s D chickens (Patient F), confirmed the hypothesis of transmission of the virus from chickens to humans. These findings suggest that transmission from human to human is not very effective. Patient B showed no sign of infection, even though he work closely with chickens, and is often in close contact with Patient A. His wife (Patient C) also tested negative in our test. The only way she could have been infected would have been through contact with her husband, since she does not deal with chickens directly.

The virus responsible for the Avian Flu is H1N5, which is a strain of the influenza viruses. 5 The virus is acquired by inhalation or ingestion of virus-

infected respiratory secretions. 1, 3, 4 The virus adheres to the epithelial cells lining the respiratory system. 1 They do so by the binding of a protein spike, found on their membrane, called hemagglutinin with sialosaccharides on the host cell surface. 1 This protein plays an important role in the specificity of the virus. For example, human influenza virus prefers to bind to the 2, 6- linked sialic acid, while avian virus prefers to bind to 2, 3-linked sialic acid. 2 Similarly, neuraminidase, present in the membrane spikes, is essential for the budding off of the virus from its host cell after replication. 5 These two proteins need to match with the host cells in order for the virus to infect them. Since the avian virus does not have high specificity for human cells, infections in humans is less acute than that in birds, however still lethal in many cases.

The specificity of these membrane proteins explains why the color seen during the Eliza test for the infected human patient (Patient D) was less intense that the color present in chickens. For similar reasons, transmission from human to human of the virus is not very effective. 1, 2 Influenza viruses have a segmented genome which makes variation between different strains possible. The two membrane proteins, hemagglutinin and neuraminidase, are encoded in different segments of its – ssRNA, therefore if two viruses happen to infect the same cell at the same time, segments from different strains can come together to form a new virus.

If this happens to be two different strains of the same virus then small mutations called antigenic drifts can occur. If the viruses are different, then the mutation accumulated is called antigenic shift. This characteristic of influenza viruses makes the development of vaccines a challenge. 5

Overtime H1N5 could build up mutations that would make it have a higher affinity for human cell, and also facilitate the mode of transmission between human to human. The high degree of variation makes it difficult for the body to build an immune response, making humans susceptible to these viruses. 5

Memos

Patient B and C: Avian Flu Test Results

To: Patient B and C

From: Livia Shehaj, Lab Assistant

Date: 5/2/2014

Dear Sir and Madam,

The result to your tests came back negative. You do not show any sign of infection from the H1N5 virus. One of the chickens from the flock Patient B works with tested positive, however. Therefore, I would like to bring to you attention that transmission from ill poultry to humans is possible. I would recommend you limit and avoid contact with chickens that seem to be infected and wear a protective mask while working, since the virus often spreads through inhalation. The virus is not known to be transmitted from human to human unless they are in very close contact. Lastly, some other suggestion that might help in preventing future infections from happening:

Wash hand before and after handing raw poultry

Use a thermometer when cooking poultry to make sure that it is cooked up to 165°F Cook eggs until the white and the egg yolks are firm

Clean carefully any utensil used to handle raw poultry3

Feel free to contact us with any questions or concerns.

Livia Shehaj

Patient D: Avian Flu Test Results

To: Patient D

From: Livia Shehaj, Lab Assistant

Date: 5/2/2014

Dear Patient D,

Your blood sample was tested for antibodies responsible for the H1N5 virus and the result came back positive. This means that you have been infected by this virus. One of the chickens from the flock you tend to tested positive. Transmission from sick poultry to humans is possible through inhalation and by eating undercooked poultry products. First, I would recommend you limit and avoid contact with chickens that seem to be infected and wear a protective mask while tending to them. Once we have taken a closer look at your symptoms we should start treatment with oseltamivir or zanamivir4, two of four prescription antiviral medications currently licensed for use in the United States.

Lastly, some other suggestion that might help in preventing future infections from happening: Wash hand before and after handing raw poultry
Use a thermometer when cooking poultry to make sure that it is cooked up to 165°F Cook eggs until the white and the egg yolks are firm
Clean carefully any utensil used to handle raw poultry
Feel free to contact us with any questions or concerns.

Livia Shehaj

References

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