

## Comparison between Haemagglutination Inhibition and Complement Fixation Tests in Detecting Antibodies Responses Following Influenza Viral Infection

Muthana, A.K. Al-zobaei

Medical Microbiology, Microbiol. Dept. Virology, College of Medicine, Univ. Anbar.

### ABSTRACT

**Objectives:** To evaluate sensitivity and specificity of HI and CF tests for detecting antibody responses following naturally occurring influenza A and B infections.

**Methods:** One hundred and twenty blood samples were drawn from one hundred patients with flue-like illness and twenty apparently healthy control subjects and tested at Central Health Laboratory in Baghdad/ Iraq using HI and CF tests for detecting three currently circulating influenza A and B strains during the period from the 1<sup>st</sup> of January 2008 to the 1<sup>st</sup> of June 2009.

**Results:** 78-80% of the patients with flu-like illness responded to at least one of three influenza virus antigens as measured by HI, while 34-35% of those patients showed a response by CF test.

**Conclusions:** HI test has a higher sensitivity and lower specificity than CF test for detecting antibody responses from patients with influenza A and B viral infections.

**Keywords :** Influenza viruses, HI, CF, Influenza serology

### INTRODUCTION

Several studies have clearly demonstrated that HI is a more sensitive method than CF test for detecting antibody responses to naturally occurring influenza virus A and B infections, Julkunen, I. *et al.* (1985); Zigler T, *et al.* (1983); Masurel A. *et al.* (1983). Haemagglutination Inhibition test detects antibodies to strain-specific haemagglutinins, whereas CF test mainly detects antibodies to type specific nucleoproteins, Julkunen, I. *et al.* (1985). Haemagglutinins are used as antigens in influenza virus, this making HI, the method of choice for measuring influenza virus infection, Madora *et al.* (1983); Palmer D.F And Whaley S.D (1986); Pyhala R. and Kleemola M. (1976); Sever J.L (1962).

The key test is HI, which depends on the ability of the virus to agglutinate human group 'O' or chicken erythrocyte and inhibition of this process by specific antibody. Diagnosis required at least a fourfold rise in antibody titer, Julkunen, I. *et al.* (1985) ;Palmer DF And Whaley

SD. (1986), whereas CF test based on fixation of complement by antigen-antibody complexes. A known amount of complement is included in the test and residual complement is detected by the addition of sheep RBCs sensitized with specific hemolysin. Absence of hemolysis indicates fixation complement and the presence of an antigen-antibody reaction, Pyhala R. and Kleemola M (1976); Sever JL (1962).

Because HI Assays for influenza virus antibodies are not widely available, clinicians often whether CF is an acceptable method for assessing the influenza infection responses of their patients. Although the point assays to monitor the influenza virus infections, search of the National Library Medicine database did not identify any reports directing comparing CF and HI for detecting influenza virus infection induce antibodies, MMWR, (2005).

The aim of this study was conducted to evaluate the HI and CF tests for measuring antibody response for influenza virus infection and to provide

comparative data for dissemination for inquiring clinicians.

### MATERIALS AND METHODS

One hundred and twenty blood samples were drawn from one hundred patients with flu-like illness in addition to twenty apparently healthy subjects as a control group during a period between 1st of January 2008 to the 1st of June 2009 in Central Health Laboratory in Baghdad /Iraq. Three ml of venous blood were taken aseptically from each patients and transferred to clean and dry plane tube (without anticoagulant) for serum collection after clotting and centrifuged the blood at 2500 rpm for 5 minutes pooled serum was kept in Eppendorf tubes at -20c. to be used.

Three strains of influenza viruses currently causing infections in the world were used: These were influenza A/Brisbane/59/2007 (H1N1)-like antigen, influenza A/Brisbane/10/2007 (H3N2)-like antigen and influenza B/Florida/4/2006-like antigen. The Haemagglutination inhibition (HI) antibody test was carried out by standard procedures, using the microtiter methods, Masurel, A. and Heijtkink AA. (1983).

Prior to HI testing, the sera were treated with cholera filtrate for 18 hours and subsequently heated at 56°C for 60 minutes, in HI test eight haemagglutination

units (8HA units) were used as antigen and reference antisera were included in each test batch. Complement fixation test was carried out by standard procedures using microtiter methods, Sever, JL. (1962). Prior to CF testing, the sera were treated with cholera filtrate for 18 hours, and subsequently heated at 56°C for 60 minutes to inactivate complement and the CF antibody titers were taken as the highest serum dilution which gave 50% fixation of complement.

### RESULTS

80% of the patients exhibit an antibody response to influenza A/Brisbane (H1N1) antigen (Table 1) and influenza A/Brisbane (H3N2) antigen (Table 2) and 78% of those patients exhibit an antibody responses to influenza B (table 3) using HI test in contrast to 18-20% of those patients exhibit negative result using the same test and this result was statistically significant ( $p < 0.01$ ), whereas the responses rates were markedly low using CF test and only 34% patients showed a respond to influenza A/Brisbane (H1N1) as showed in (Table 1), 35% patients showed respond to influenza A/Brisbane (H3N2) using the same test (Table 2), and only 21% of the patients respond to influenza B/Florida/4/2006 like antigen (Table 3).

Table 1: Sensitivity and specificity of HI and CF tests for detecting influenza A /Brisbane/59/2007 (H1N1)-like antigen.

Test used		Complement fixation		Total
		+	-	
Haemagglutination inhibition	+	31	49	80
	-	3	17	20
Total		34	66	100
Sensitivity = 91.2% Specificity = 25.7%		Sensitivity = 38.7% Specificity = 85%		

Table 2: Sensitivity and specificity of HI and CF tests for detecting influenza A /Brisbane/10/2007 (H3N2)-like antigen.

Test used		Complement fixation		Total
		+	-	
Haemagglutination inhibition	+	35	45	80
	-	0	20	20
Total		35	65	100
Sensitivity = 100% Specificity = 30.8%		Sensitivity = 43.7% Specificity = 100%		

Out of 80% patients exhibiting HI response to influenza A/Brisbane (H1N1), only 31 patients showed CF response to these strains (table 1), at the same time out of the 20 patients non exhibiting response to these strains, only three of them showed CF response. Out of 80 patients exhibiting an HI (H3N2), only 35 patients showed CF response to these strains, at the same time

20% of the patients not response to HI (table 2). Out of 35% patients exhibiting HI response to influenza B, only one patient showed CF response to influenza B and 22 of the patients exhibiting non HI influenza B, 21 of them response to influenza B (Table3). All healthy control sera were negative for HI and CF test against three strains of influenza viruses.

Table 3: Sensitivity and specificity of HI and CF tests for detecting influenza B /Florida/4/2006 -like antigen.

Test used		Complement fixation		Total
		+	-	
Haemagglutination inhibition	+	35	43	78
	-	1	21	22
Total		36	64	100
Sensitivity = 97.2% Specificity = 32.8%		Sensitivity = 44.9% Specificity = 95.4%		

**DISCUSSION**

Several studies have shown that the incidence of antibodies against three currently strains of influenza viruses can be measured by sero-epidemiological studies using blood samples collected from patients with flu-like illness MMWR (2009); MMWR (2008). Sera collected and treated with 56°C to inhibit complement and this finding was agreement with, Okuno, Y. *et al.* (1990).

Results in the present study showed that Iraqi persons were infected with influenzaA/Brisbane/59/2007(H1N1)-like antigen, influenza A/Brisbane /10/2007 (H3N2)-like antigen and influenza B/Florida/4/2006-like antigen but the titer of antibody was differ for each strain in spite of the same HA units (8HA units) used in this test as reported by previous studies, Madora, H. *et al.* (1983); Pyhala R. and Kleemola (1976). and this might be interpreted that each of influenza virus isolates were inhibited with different titers of antibodies in HI and CF test and this was provided an evidence for prevalence strains of influenza virus in the world and this was in agreement with the result reported by WHO, MMWR, (2009); MMWR, (2008). Who indicated that these strain was circulating in population during 2008-2009 season.

Using of HI for detecting antibodies against different strains of influenza A and B in the sera of the patients and healthy control

group was consistent with other studies, Julkunen, I., *et al.* (1985); Madora H., *et al.* (1983); Pyhala R. and Kleemola M.(1976) who reported that this test was more sensitive for detecting antibody responses to naturally occurring influenza A and B viruses and also chosen of CF test in this research support HI in diagnosis of influenza viral infection, Julkunen, I., *et al.* (1985); Zigler T., *et al.* (1983) ;Azad TM., *et al.* (2004).

Detection of antibody responses to naturally and experimentally occurring influenza A and B viruses using HI and CF test was consistent with other reports, Zigler, T. *et al.* (1983); Madora H., *et al.* (1983).

From this study we can conclude that HI testing is superior to CF testing for detection antibody responses of patients infected with influenza viruses and it's clear that the CF assays give false negative antibody responses results for the majority of patients infected with influenza viruses and influenza A/Brisban/59/2007 (H1N1)-like antigen, influenza A/Brisban/10/2007 (H3N2)-like antigen and influenza B/Florida/4/2006 like antigen was circulating strains during 2008-2009 season.

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## ARABIC SUMMARY

مقارنة بين تقنيتي اثباط التلازن الدموي وتثبيت المتمم في تشخيص استجابة الاجسام المضادة بعد الاصابة بفايروس الأنفلونزا

مثنى علي خليل الزوباعي

كلية الطب-قسم الاحياء المجهرية – جامعة الانبار

**الأهداف:** تقييم حساسية وخصوصية تقنيتي اثباط التلازن الدموي وتثبيت المتمم في تشخيص استجابة الاجسام المضادة بعد الاصابة الطبيعية بفايروس الأنفلونزا نوعي

**الطرائق:** اجريت هذه الدراسة بعد سحب مئة وعشرون نموذج دم منهم مئة من مرضى يعانون من اعراض انفلونزا اضافة الى عشرون من مجموعة سيطرة اصحاء في مختبر الصحة المركزي في بغداد-العراق حيث طبقت تقنيتي اثباط التلازن الدموي وتثبيت المتمم على جميع العينات في آن واحد لتشخيص الاصابة بثلاث عترات منتشرة حديثا لفايروس الأنفلونزا خلال الفترة المحصورة بين الاول من كانون الثاني 2008 الى الاول من حزيران 2009

**النتائج:** اظهرت نتائج الدراسة ان 78-80% من المرضى الذين يعانون من اعراض الانفلونزا يستجيب على الاقل لواحد من ثلاث مستضدات من فايروس الانفلونزا باستخدام تقنية اثباط التلازن الدموي ،بينما اظهر 34-35% من هؤلاء المرضى استجابة عند استخدام تقنية تثبيت المتمم .

**الاستنتاج:** تقنية اثباط التلازن الدموي تمتلك حساسية عالية وخصوصية واطنة مقارنة بتقنية تثبيت المتمم في تشخيص استجابة الاجسام المضادة للإصابة بفايروس الأنفلونزا نوعي.