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Comparison of Complement Fixation and Hemagglutination Inhibition Assays for Detecting Antibody Responses following Influenza Virus Vaccination

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Complement fixation (CF) was compared to hemagglutination inhibition (HI) as a method for identifying antibody responses to influenza virus vaccination. CF assays were performed at two different laboratories using paired (pre- and postvaccination) sera from 38 vaccinated laboratory employees; HI assays were performed at a third laboratory. As expected, most vaccinees (31/38 = 82%) responded to at least one of three influenza virus antigens as measured by HI. In contrast, only 21% (8/38) of vaccinees showed a response by CF at laboratory 1, and only 29% (11/38) showed a response by CF at laboratory 2. These findings indicate that due to low sensitivity, CF assays should not be used to assess the antibody response to influenza virus vaccination.

Several studies have clearly demonstrated that hemagglutination inhibition (HI) is a more sensitive method than complement fixation (CF) for detecting antibody responses to naturally occurring influenza virus A and influenza virus B infections (4, 6, 8). HI detects antibodies to strain-specific hemagglutinins, whereas CF mainly detects antibodies to type-specific nucleoproteins (9). Hemagglutinins are used as antigens in influenza virus vaccines, thus making HI the method of choice for measuring vaccine-induced antibodies (1, 2, 3, 5).

Because HI assays for influenza virus antibodies are not widely available, clinicians often ask whether CF is an acceptable method for assessing the influenza virus vaccine responses of their patients. Although the points mentioned above argue against the use of CF assays to monitor vaccine responses, a search of the National Library of Medicine database did not identify any reports directly comparing CF and HI for detecting influenza virus vaccine-induced antibodies. We thus conducted a study to verify that HI is more sensitive than CF for measuring antibody responses to influenza virus vaccination and to provide comparative data for dissemination to inquiring clinicians.

Subjects. Study participants included 38 Focus Technologies employees electing to receive influenza virus vaccination in December 2001 (vaccinees) and 11 employees electing not to receive the vaccine (controls). All study participants were between 19 and 63 years of age and provided informed consent for specimen collection. Vaccinees donated a prevaccination blood specimen and then received the 2001-to-2002 trivalent influenza virus vaccine (Aventis Pasteur, Swiftwater, Pa.) 1 to 12 days later (median, 7 days). The vaccine contained the hemagglutinins of influenza virus A/New Caledonia/20/99 (H1N1), influenza virus A/Panama/2007/99 (H3N2), and influenza virus B/Victoria/504/2000. Vaccinees then donated a second blood specimen 16 to 32 days (median, 21 days) following vaccination. Two blood specimens were also obtained from controls; the time between collection of the two samples ranged from 24 to 28 days (median, 26 days). None of the vaccinees or controls self-reported influenza virus infection in the 4 months following the donation of the blood samples. All blood specimens were processed within 8 h of collection, and the serum was stored in 1.0-ml aliquots at −70°C. All sera were coded such that individuals performing HI and CF assays were unaware of the donors' vaccination status.

HI. Using a starting dilution of 1:5, serum HI titers to the vaccine components were measured by Retroscreen Virology Ltd. (London, United Kingdom) as previously described (1). Coded sera were tested in run sizes of 8 to 20 samples following a plan designed by the authors; paired sera from a given study participant were tested on the same assay run. A positive response was defined as a fourfold increase in titer between pre- and postvaccination sera; a titer of <5 was assigned a titer value of 2.5.

CF. Using a starting dilution of 1:8, serum CF titers to influenza virus A and influenza virus B (hereafter referred to as A and B, respectively) (BioWhittaker, Walkersville, Md.) were measured following established procedures in two different laboratories (7). Coded sera were tested in run sizes of 8 to 20 samples as outlined by the authors; paired sera from a given study participant were tested on the same assay run. A positive response was defined as a fourfold increase in titer between pre- and postvaccination sera; a titer of <8 was assigned a titer value of 4.

As shown in Table 1, 31/38 (82%) vaccinees exhibited an antibody response to at least one influenza virus antigen as assessed by HI. Although 2/11 (18%) unvaccinated controls also showed a response to A by HI, the difference between the proportions (82 versus 18%) was statistically significant (defined [using contingency table analysis] as $P < 0.01$). In contrast to the high rate of responses noted in the vaccinee group by HI, the response rates as assessed by CF were markedly lower. Only 8/38 (21%) vaccinees showed a response to A and/or B as assessed by CF testing performed at laboratory 1, and only 11/38 (29%) showed a response by CF testing performed at laboratory 2. Both of these proportions were statis-

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TABLE 1. HI and CF response patterns following influenza virus vaccination[a]

Antibody response pattern by HI	Total no. of samples	No. of donor samples exhibiting the indicated CF response pattern at:							
		Laboratory 1				Laboratory 2			
		Neither A nor B	A only	B only	A and B	Neither A nor B	A only	B only	A and B
Neither	7	7	0	0	0	6	0	0	1
B	2	2	0	0	0	2	0	0	0
A	13	9	1	2	1	9	2	2	0
	16	12	0	3	1	10	0	2	4
Both	38	30	1	5	2	27	2	4	5

[a] Antibody response patterns were as follows: neither, negative for A-NC and/or A-Pan as well as for B; B, positive for B but negative for A-NC and/or A-Pan; A, positive for A-NC and/or A-pan but negative for B; both, positive for A-NC and/or A-Pan as well as for B. A-NC, influenza virus A/New Caledonia; A-Pan, influenza virus A/Panama.

tically different from the proportion observed for HI testing; however, the proportion of vaccinees showing a response by CF at laboratory 1 and the proportion showing a response by CF at laboratory 2 (i.e., 21 versus 29%) did not significantly differ. None of the 11 controls showed a response by CF (data not shown).

Table 2 presents a more detailed breakdown of CF responses at laboratories 1 and 2 for the two most common HI response patterns. Of the 13 donors exhibiting an HI response to A but not B, only one donor showed a CF response to A alone at both laboratory 1 and laboratory 2. Interestingly, two donors in this HI response group (HI response to A alone) showed a CF response to B at both laboratory 1 and laboratory 2. The explanation for this inconsistency between HI and CF responses remains unclear.

TABLE 2. CF response patterns in selected HI response pattern groups

Antibody response by HI	CF response				No. of donor samples[a]
	Laboratory 1		Laboratory 2		
	A	B	A	B	
Positive for A but not B[a]	No	No	No	No	8
	No	No	Yes	No	1
	Yes	No	Yes	No	1
	No	Yes	No	Yes	1
	Yes	Yes	No	Yes	1
	No	Yes	No	No	1
Positive for A and B[a]	No	No	No	No	8
	No	No	No	Yes	2
	No	No	Yes	Yes	2
	Yes	Yes	Yes	Yes	1
	No	Yes	No	No	2
	No	Yes	Yes	Yes	1

[a] The number of donor samples testing positive for A but not B in HI assays was 13; the number of donor samples testing positive for A and B in HI assays was 16.

Of the 16 donors exhibiting an HI response to both A and B, only one donor showed a CF response to both A and B at both laboratory 1 and laboratory 2. Of the remaining 15 donors, most ($n = 8$) showed no CF response to A or B at either laboratory 1 or laboratory 2.

In summary, these findings show that HI testing is superior to CF testing for the detection of antibody responses following influenza virus vaccination. Although minor differences in responses assessed by CF were observed at two different laboratories, it is clear that CF assays give false-negative antibody response results for the majority of vaccinees.

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