Performance of Rapid Tests for Detection of Avian Influenza A Virus Types H5N1 and H9N2

Among the avian influenza A virus subtypes, the H5N1 and H9N2 viruses have the potential to cause an influenza pandemic because they are widely prevalent in avian species in Asia and have demonstrated the ability to infect humans (8). Currently, human infections with wild-type (wt) strains of these viruses could occur in the United States in poultry and turkey farm workers and in travelers returning from countries in which avian influenza viruses are prevalent in birds, such as Thailand, Vietnam, and China. Laboratory-acquired infections could also occur in vaccine researchers working with wt or candidate vaccine viruses, including cold-adapted (ca) viruses (2, 3, 11). Published reports indicate that the Directigen Flu A antigen capture enzyme immunoassay (Becton Dickinson, Sparks, MD) can detect H5N1, H7N2, and H7N3 avian influenza viruses and that a DAKO direct fluorescent antibody reagent (DAKO, Cambridgeshire, United Kingdom) can detect H5N1 avian influenza virus, although these commercial rapid antigen tests are insensitive (1, 4, 6, 13, 14). We sought to determine if rapid assays routinely used in clinical microbiology laboratories, i.e., the shell vial assay and two commercial antigen capture enzyme immunoassays, could detect wt and ca avian influenza A virus types H5N1 and H9N2.

wt and ca influenza A viruses derived from A/Vietnam/1203/2004 (H5N1) and A/chicken/Hong Kong/G9/97 (H9N2) were used to determine if the shell vial assay, Directigen Flu A+B (Becton Dickinson), and x/pect Flu A&B (Remel Lenexa, KS) could detect avian influenza A viruses (2, 10). The Directigen Flu A+B and x/pect Flu A&B kits were used to test serial 10-fold dilutions (10 to 10^6 50% tissue culture infective doses [$TCID_{50}$]) of the A/Hong Kong/491/1997 wt and A/Indonesia/05/05 wt strains and of the A/Vietnam/1203/2004 wt strain of H5N1 influenza A virus. The shell vial assay was performed as previously described but without centrifugation of the inoculated shell vials, because the appropriate holders for the centrifuge in the biosafety level-3 (BSL-3) facility were not available (5). We used A549 cells and RhMK cells (Diagnostic Hybrids, Inc., Athens, OH) and Bartels influenza A monoclonal antibody (Trinity Biotech, Wicklow, Ireland). Shell vial coverslips were fixed for staining after 1, 2, and 5 days of incubation at 32°C. The shell vials inoculated with the wt and ca viruses were incubated at 32°C, a temperature that is permissive for the cold-adapted viruses and for wt viruses as well. The Directigen Flu A+B and x/pect Flu A&B assays were performed by following the manufacturers' instructions. Shell vial culture assays and the enzyme immunoassays were performed in duplicate. The wt and ca influenza A viruses were propagated in the allantoic cavity of embryonated eggs. All manipulations with live virus were performed in a USDA-approved BSL-3 facility.

At concentrations of 20 and 2×10^4 $TCID_{50}$, H5N1 ca virus was detected after 1, 2, and 5 days of incubation equally well in both A549 and RhMK cells. The H5N1 wt virus at 2×10^4 $TCID_{50}$ was also detected in both cell lines, but at 20 $TCID_{50}$ the wt virus was detected only at 5 days in A549 cells and at 2 and 5 days in RhMK cells. The H9N2 wt virus was detected at both concentrations after 1, 2, and 5 days of incubation equally well in both cell lines. The H9N2 ca virus was also detected in both cell lines at 2×10^4 $TCID_{50}$, but at 20 $TCID_{50}$ the ca virus was detected only at 5 days in A549 cells and at 2 and 5 days in RhMK cells. Although this experiment was carried out at an incubation temperature of 32°C, we have previous unpublished experimental data showing that the wt H5N1 viruses replicate equally well at 32°C and 37°C.

Table 1 shows the results obtained with the two antigen capture enzyme immunoassays with both concentrations of wt and ca H5N1 and H9N2 influenza A viruses. Neither assay detected virus at 50 $TCID_{50}$, but H9N2 wt and ca viruses were detected by both assays at 5×10^4 $TCID_{50}$. H5N1 ca virus was detected at 5×10^4 $TCID_{50}$ in both assays, but the wt virus was not detected in either assay at this concentration. These data generated the concern that additional strains of wt avian influenza viruses may not be detected by the antigen capture enzyme immunoassays. When serial dilutions of A/Vietnam/1203/2004 (H5N1) wt virus were tested, both assays gave a positive result only with a very high concentration of virus (10^6 $TCID_{50}$) and even then, the Directigen result was weakly positive. The A/Indonesia/05/05 (H5N1) wt virus gave a weak positive result with the x/pect test and a negative result with the Directigen kit when tested at a concentration of 10^5 $TCID_{50}$. Both assays were positive with A/Indonesia/05/05 (H5N1) wt at 10^6 $TCID_{50}$. The A/Hong Kong/491/1997 (H5N1) wt virus was not detected by either assay at any of the concentrations tested.

Peak concentrations of wt human influenza A viruses in nasopharyngeal wash specimens occur at about 48 h postinfection and range from 10^3 to 10^7 $TCID_{50}$/ml, with a positive correlation between the amount of virus shedding and the severity of the clinical response (12). We tested the shell vial assay and the two antigen capture assays with avian influenza virus titers below and within this range. The shell vial assay was able to detect virus at both concentrations, although results for H5N1 wt and H9N2 ca viruses required 2 to 5 days to become positive with low-titer inoculum. The Directigen Flu A kit has been reported to have a limit of detection for free virus of 1.63×10^3 infectious virus particles (9). Kaiser et al. reported that with nasopharyngeal wash samples from volunteers inoculated with influenza A virus H1N1, the Directigen Flu A assay-positive samples had a mean virus titer of 2.9×10^4 $TCID_{50}$/ml, and negative samples had a mean virus titer of 2.45×10^2 $TCID_{50}$/ml (7). We found that both the Directigen and x/pect assays gave positive results when the samples contained 5×10^4 $TCID_{50}$ of wt and ca avian influenza A virus type H9N2 and the A/Vietnam/1203/2004 (H5N1) ca virus. The H5N1 wt viruses A/Vietnam/1203/2004 and A/Indonesia/05/05 were clearly positive in both assays at 10^6 $TCID_{50}$, but the A/Hong Kong/491/1997 wt virus was not detected. The package inserts for both the Directigen and x/pect assays indicate they can detect H5N1 and H9N2 viruses from cell culture. Previous reports indicate that H5N1 wt viruses can be detected in human and avian specimens using the Directigen assay. Ryan-Poirier et al. proposed that the Directigen Flu A assay can detect cell-associated virus more readily than free virus (9). These data and those reported by Kaiser et al. lead us to believe that the antigen capture assays may be useful for detection of both wt and ca H5N1 and H9N2 influenza A viruses

TABLE 1. Performance of antigen capture enzyme immunoassays for detection of avian influenza A virus types H5N1 and H9N2

Expt no.	Virus	Virus titer in sample tested ($TCID_{50}$/ml)	Result by enzyme immunoassay[a]	
			Directigen	x/pect
1	A/Vietnam/1203/2004 (H5N1) wt	50	−	−
		5×10^4	−	−
	A/Vietnam/1203/2004 (H5N1) ca	50	−	−
		5×10^4	+	+
	A/chicken/Hong Kong/G9/97 (H9N2) wt	50	−	−
		5×10^4	+	+
	A/chicken/Hong Kong/G9/97 (H9N2) ca	50	−	−
		5×10^4	+	+
2	A/Vietnam/1203/2004 (H5N1) wt	10	−	−
		10^2	−	−
		10^3	−	−
		10^4	−	−
		10^5	−	−
		10^6	+*	+
	A/Hong Kong/491/1997 (H5N1) wt	10	−	−
		10^2	−	−
		10^3	−	−
		10^4	−	−
		10^5	−	−
		10^6	−	−
	A/Indonesia/05/2005 (H5N1) wt	10	−	−
		10^2	−	−
		10^3	−	−
		10^4	−	−
		10^5	−	+*
		10^6	+	+

[a] *, weakly positive.

in nasopharyngeal washes from symptomatic patients early in their infections if the level of virus replication exceeds 10^3 or 10^4 $TCID_{50}$ (7, 9). Although the highest viral loads of human influenza A viruses are found in nasal wash samples, avian influenza A H5N1 may be present at higher titers in the throat, and thus a throat swab sample may be a better specimen for diagnosis (13). Our data also demonstrate that Bartels influenza monoclonal antibody can be used to detect avian influenza A virus types H5N1 and H9N2. Positive specimens from suspect patients should be sent to state public health laboratories or the Centers for Disease Control and Prevention for virus characterization.

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