An Improved Hemagglutination-Inhibition Test for Pseudorabies Virus

Nobuhiro TETSU, Yuji INABA, Masayoshi YUKAWA¹⁾, Shigeo OHBA²⁾, Ken-ichi YOSHIKI³⁾, Tadashi HIRAHARA³⁾, Akihiro IZUMIDA³⁾, Yoshito FURUYA⁴⁾, and Naoshi ITOH⁴⁾

Departments of Veterinary Epizootiology, ¹⁾Biomedical Science, and ²⁾Veterinary Internal Medicine, College of Agriculture and Veterinary Medicine, Nihon University, Fujisawa, Kanagawa 252, ³⁾Biken Laboratory, Uji, Kyoto 611, and ⁴⁾Animal Health Research Laboratory of Chiba Prefecture, Sakura, Chiba 281, Japan

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Pseudorabies virus (PrV), a member of the family Herpesviridae, also known as suid herpesvirus 1 and Aujeszky's disease virus, is a significant cause of morbidity, and an economically important pathogen of the swine population. Hemagglutination (HA) by some herpesviruses, such as bovine herpesvirus 1 [9], equid herpesvirus 1 (equine abortion virus) [3, 4], feline herpesvirus 1 [2, 5] and infectious laryngotracheitis virus [7], has been reported. We have also reported that PrV agglutinates mouse erythrocytes and the HA reaction can be inhibited by specific antiserum [8]. In the present study we found that a modification of the HA-inhibition test gave an improvement of the sensitivity of this test compared to the conventional test.

CPK cells derived from pig kidney were prepared as described previously [6, 8] and used for preparation of HA antigens and virus titration.

The YS-81 strain [1] of PrV, obtained from National Institute of Animal Health, Tsukuba, Ibaraki, Japan, was clarified by centrifugation and stored at -80°C . The culture fluid was also used as HA antigen.

HA and HI tests were carried out by the microtiter method as described previously [8]. The diluent used for the HA and HI tests was Dulbecco's phosphate buffered saline (0.15 M NaCl, 0.02 M phosphate buffer, pH 7.2) containing 0.2% bovine serum albumin and 0.001% gelatin. Mouse, strain BALB/C, erythrocytes were used in 0.3% suspension. In HA tests, serial 2-fold dilutions of HA antigen were prepared in 0.025 ml amounts, and mixed with 0.05 ml of erythrocyte suspension after adding 0.025 ml of the diluent. The mixtures were incubated at 25°C for 2 hr before the results were read. The HA titer was expressed as the reciprocal of the highest antigen dilution showing complete HA.

In HI tests, 0.1 ml of the serum and 0.3 ml of the diluent were mixed, inactivated at 56°C for 30 min and mixed with 0.5 ml of 25% kaolin to remove nonspecific inhibitors. After shaking for 1 hr at 25°C , kaolin was removed by centrifugation at 1,000 g for 10 min, and the supernatant fluid was mixed with 0.1 ml of packed erythrocytes. After incubation at 37°C for 1 hr, the cells were removed and the supernatant fluid was used for HI test as the 8-fold dilution of the serum. Four units of HA antigen in 0.025 ml and 0.025 ml of serial 2-fold dilutions of the treated serum were mixed and the mixtures were incubated at 37°C for 1 hr, or at 4°C for 24, 48 or 72 hr, as indicated in each experiment. Each mixture then received 0.05 ml of 0.3% erythrocyte suspension, incubated at 25°C for 2 hr and the results were read. The HI antibody titer was expressed as the reciprocal of the highest serum dilution showing complete HI. HI antibody titers of 8 or higher were taken as positive.

Infectivity assay was carried out in tube cultures of CPK cells. The inoculated cultures were examined for cytopathic effect (CPE) after incubation at 37°C for 4 days and the TCID₅₀ titer was calculated.

In neutralization (NT) tests, serial 2-fold dilutions of the serum inactivated at 56°C for 30 min were made in maintenance medium and 0.2 ml of each dilution was mixed with an equal volume of virus suspension containing 200 TCID₅₀/ml. After incubation at 37°C for 1 hr, 0.1 ml of the virus-serum mixtures was inoculated into each of two tube cultures, and then examined for CPE. The NT antibody titer was expressed as the highest serum dilution which showed complete inhibition of CPE in at least one of the two tubes. NT antibody titers of 2 or higher were taken as positive.

Two pigs, 5 weeks of age, were inoculated subcutaneously with $10^{8.5}$ TCID₅₀ of PrV. Weekly serum samples were collected and tested for HI antibody titer by incubation of antigen-serum

Table 1. Effect of incubation time of antigen-serum mixture on HI antibody titer of sera from infected pigs

Pig No.	Incubation	Weeks after virus inoculation					
		0	1	2	3	4	5
16	37°C, 1 hr	<8 ^{a)} (<2) ^{b)}	<8(<2)	< 8(<8)	< 8(8)	8 (8)	8 (32)
	4°C, 24 hr	<8	<8	< 8	8	8	8
	4°C, 48 hr	<8	<8	< 8	8	32	32
	4°C, 72 hr	<8	<8	< 8	8	32	32
107	37°C, 1 hr	<8 (<2)	<8 (16)	< 8 (32)	< 8 (32)	8 (32)	8 (32)
	4°C, 24 hr	<8	<8	< 8	8	8	8
	4°C, 48 hr	<8	8	16	32	64	64
	4°C, 72 hr	<8	8	16	32	32	32

a) HI antibody titer.

b) NT antibody titer.

mixture at 37°C for 1 hr, which was established as a standard method in the previous paper [8], or at 4°C for 24, 48 or 72 hr. These serum samples were also tested for NT antibody titer. As shown in Table 1, the standard HI test for all of the pigs became positive 4 weeks after virus inoculation. The HI tests for pig No. 16 by incubation of antigen-serum mixture at 4°C for 24, 48 or 72 hr was positive 3 weeks after virus inoculation, respectively, and for pig No. 107 became positive 3 weeks, and 1 week after virus inoculation, respectively. The modified HI tests as well as the NT test was positive 1 up to 3 weeks earlier than the standard HI test. At 4 weeks after virus inoculation, HI antibody titers by the modified method (4°C, 48 or 72 hr) were 4- to 8-fold higher than those by the standard method. The serum samples obtained from these pigs before infection were negative by all the tests.

Serum samples obtained from 6 pigs, 5 weeks of age, experimentally infected with PrV were tested for HI antibody titer by the standard incubation (37°C, 1 hr) and by the modified incubation (4°C, 48 hr). These serum samples were also tested for NT antibody titer. As shown in Table 2, all of the pigs were negative for HI antibody by both tests before infection. High-titered HI antibody was detected by the modified method after infection in all the animals. Marked enhancement (4- to 8-fold) in HI antibody titer at 4 weeks after infection was demonstrated in all the animals by the modified incubation.

A total of 60 serum samples collected from pigs in 2 epizootic areas of pseudorabies were tested for HI antibody titer by the standard incubation

Fig. 1. Correlation between HI antibody titer obtained by the modified method and that by the standard method in individual serum samples from 60 pigs in 2 epizootic areas of pseudorabies.

(37°C, 1 hr) and by incubation at 4°C for 48 hr, and for NT antibody titer. As shown in Fig. 1, the modified HI test proved to be more sensitive: all of the serum samples having NT antibody were

Table 2. Comparison of HI antibody titer obtained by the modified method (4°C, 48 hr) and that by the standard method (37°C, 1 hr)

Pig No.	Incubation	Weeks after virus inoculation	
		0	4
1	37°C, 1hr	<8 ^a (<2) ^b	16(32)
	4°C, 48hr	<8	128
2	37°C, 1hr	<8(<2)	16(32)
	4°C, 48hr	<8	128
3	37°C, 1hr	<8(<2)	32(16)
	4°C, 48hr	<8	128
16	37°C, 1hr	<8(<2)	8(8)
	4°C, 48hr	<8	32
84	37°C, 1hr	<8(<2)	128(64)
	4°C, 48hr	<8	512
107	37°C, 1hr	<8(<2)	8(32)
	4°C, 48hr	<8	64

a) HI antibody titer.

b) NT antibody titer.

positive by this method, while the standard method gave negative results with 3 samples which had HI antibody titers of 8 to 16 by the modified method. Of the remaining 57 samples, 37 had HI antibody titers 2 to 32 times higher by the modified method than the standard method, while 20 samples, including 11 samples which were negative by the NT test, had the same titers.

All these findings indicate that the sensitivity of the HI test for PrV is significantly improved by incubation of antigen-serum mixture at 4°C for 48 hr.

As to the reason why an enhancement in HI antibody titer occurs by a prolonged incubation at 4°C, it can be thought that the avidity of HI antibody for antigen is so low for a considerable time after incubation of antigen-serum mixture.

The modified HI test had a sensitivity and a specificity comparable to that of the NT test and appeared to be somewhat more sensitive than the NT test (Tables 1 and 2).

The increased sensitivity was particularly useful for the serodiagnosis of pseudorabies in swine.

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要 約

仮性狂犬病ウイルスの改良赤血球凝集抑制試験（短報）：鐵 伸弘・稲葉右二・湯川真嘉¹⁾・大場茂夫²⁾・吉木研一³⁾・平原 正³⁾・出水田昭弘³⁾・古屋美人⁴⁾・伊藤尚志⁴⁾（日本大学農獣医学部獣医伝染病学研究室，¹⁾実験動物学研究室，²⁾獣医内科学研究室，³⁾微生物化学研究所，⁴⁾千葉県家畜衛生研究所）——仮性狂犬病（オーエスキー病）ウイルス（プタヘルペスウイルス 1）の赤血球凝集抑制（HI）試験で，抗原-抗体混合物を4℃，48時間感作すると，一般的な37℃，1時間感作よりも実験感染豚血清では4～8倍，本病流行地の豚血清ではそのほとんどが2～32倍高い HI 抗体価を示した．以上の成績から，4℃，48時間感作 HI 試験は本病の血清診断として特に有効である．