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LETTER TO EDITOR

AN IMPROVED AGAR GEL PRECIPITIN (AGP) TEST MEDIUM FOR THE DETECTION OF AVIAN ENCEPHALOMYELITIS ANTIBODIES

SIR: An agar medium has been improved for the detection of precipitating antibodies to avian encephalomyelitis (AE) in chickens. It is recommended that Bacto agar (Difco, USA) is used as a source of agar at 8% NaC1 in 0.01M Tris-HC1 buffer solution (PH 8.0).

AE AGP antigen was prepared using six-day-old embryonated eggs inoculated via the yolk sac with 10^2 to 10^3 EID_{50} of AEV-VR strain obtained from the Association of Biological Products for Animal Health, Tokyo, Japan. At ten days post-inoculation, infected brains and intestines were harvested, pooled and homogenised separately without adding any media. All AGP antigens were kept at -80°C prior to use.

Comparative studies were carried out on the effects of pH and NaC1 concentrations on AGP test using phosphate buffer solution (PBS: 0.01M NaH_2PO_4, 0.01M Na_2HPO_4). Precipitation lines were produced clearly at pH 7.5 and 8.0 while no precipitation lines were formed at lower pH (6.5 and 7.0). In addition, a precipitation line was formed at the portion close to the antigen at 15% NaC1, and in the case of 80% NaC1, at the central portion between the antigen and the antisera.

Two kinds of basic buffer solutions used for the AGP agar media preparation were compared. Alteration in pH of the buffer solutions were determined on adding different concentrations of NaC1. The basic buffer solution (0.01M) ranging from pH 6.5 to 8.5 were prepared using PBS and Tris-HC1 buffer solution (TBS). Subsequently, 8% or 15% NaC1 were added to these buffer solutions and the pH measured. In PBS, the pH dropped greatly after adding NaC1. On the other hand, in TBS, the pH is increased slightly after adding 8% NaC1 and is relatively stable even after adding 15% NaC1.

Finally, it was decided to use Bacto agar as a source of agar at 8% NaC1 in 0.01M TBS (pH 8.0). This improved agar medium was used for the detection of AGP antibodies against AEV of sera from the field using the improved agar medium as compared to the conventional agar medium (Bacto agar, 8% NaC1 in PBS pH 7.4).

Preliminary field trials showed that all sera from flocks previously vaccinated for AE exhibited precipitin lines using this improved agar medium. However, no precipitin lines were observed using the conventional agar medium (Table 1).

TABLE 1

Detection of AGP antibodies against AEV of sera from the field using the improved agar medium as compared to the conventional agar medium

Farm	Age at vaccination (weeks)	Age at serum collection (weeks)	Results	
			Conventional agar medium	Improved agar medium
A	10	69	*0/10	10/10
B	14	43	0/5	5/5
C	15	22	0/5	5/5
D	17	69	0/5	5/5

*No. positive/No. tested

Ikeda (1977) used concentrated tissue extracts from AEV infected embryos as the antigen. Lukert and Davis (1971) reported that AEV infected gastrointestinal tract reacted positively with antiserum, but infected brain tissue did not yield a good precipitin antigen. In this study, unconcentrated brain tissues were used as an antigen with good results. On the other hand, intestinal tract did not react with antisera (data not shown). In addition, the homogenate made from brain tissues consistently had about ten times the virus titer of homogenate from intestinal tissues (Matsumoto and Murphy 1977). It may be reasonable to use the infected brain as an antigen for the AE AGP test. Studies using this improved medium of AGP test is in progess to evaluate the immune status of the breeder flocks and also to detect for presence of field strain of AEV infections in layer flocks in Malaysia.

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