Preparation and Evaluation of Stained Pertussis Antigen for Serodiagnosis of Whooping Cough

Short Communication

Afsharpad[*1], K., Mohammadi, A.[2] and Mirchamsy, H.[2]

1. Human Bacterial Vaccines Research & Production Dept., Razi Vaccine & Serum Research Institute, P.O.Box:11365-1558, Tehran, Iran

2. Human Viral Vaccines Research & Production, Razi Institute

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Summary

For rapid clinical diagnosis and also determining the immune response of healthy persons to whooping cough a stained pertussis antigen was prepared. In this regard the 48h culture of *Bordetella pertussis*, Tohama wild strain, on methyl cellulose enrichment medium (B2) was used. The medium was supplemented with methyl cellulose, Rosebangal as vital color and phosphate buffer containing Thiomersal (1/5000). A comparative study with *Bordetella pertussis*/toxin IgG ELISA has shown that the local antigen is highly specific and sensitive. Therefore the antigen may provide a diagnostic laboratory tool in epidemiological study.

***Key word*:** agglutinogen, antigen, antibody, pertussis

Introduction

Woopind cough is a highly contagious respiratory infection due to a gram negative cocobacilli, *Bordetella pertussis* (Bemis & Burn 1994). However, the disease is an acute and highly communicable infection equally affecting children and adults, it is symbolically classified as a common childhood infection. WHO estimates the total number of pertussis cases is responsible for half a million to one million deaths annually (Muller *et al* 1986). A combination of antibiotics and serum suggests for

*Author for correspondence. E-mail:afsharpad@hotmail.com

treatment of the disease but rapid diagnosis is very important. The laboratory diagnosis of pertussis is based on the direct culture of nasopharyngieal specimen during the incubation period and catarrhal stages of disease (Kwantes *et al* 1983). Pathological and bacteriological data indicated that isolation of bacteria from nasopharyngeal discharge is possible just at the first week of infection and attack stage of the disease and only from the end of lung alveoli (Kwantes *et al* 1983, Mirchamsy 1997, Ryan 1997). The routine serological test by using especial bacterial antigen is not economic (Ruuskamen *et al* 1991). A cold chain condition and using %20CosH as stabilizer is necessary for antigen production. In order to detect the adult infection, which appears due to the vaccine failure (He *et al* 1994, Hewlett 1992, Keitel & Edwards 1995) and also for evaluation of children protection against the infection a reliable and reproducible tool would be necessary. This problem has generated a considerable interest in our laboratory for producing a stained pertussis antigen evaluated by standard serum and agglutinogen.

Materials and Methods

Virus strain. Four pertussis strains including 134 and 509 (received from Rijks Institute, The Netherlands), 18323 and Tohama wild strain were used.

Stained pertussis antigen (SPA) preparation. SPA was prepared according to Alton & Jones (1988) method. Briefly, 72h culture of each *B.pertussis* strains (134, 506, 18323 and Tohama wild strain) was prepared in phosphate buffer solution (PBS, containing 1/5000 Thiomersal pH7.2) by magnetic stirrer. 1ml of Rosebengal solution (1%) was added to 35ml of the above suspension and incubated at 4°C for 24h. Then the suspension was centrifuged at 5000rpm for 20min. The supernatant was discarded and 12ml of PBS per each gram of packed cell was added. The suspension was homogenized and using Hopkines tube regulated at the level of 8% of bacterial germ, the final suspension was prepared. In another study, a mixture of the homogenized suspension of 72h cultures of 134 and 509 pertussis strains was

stained in a similar way. WHO standard pertussis antiserum (Statens Serum Institut *B.pertussis* ATOX//220681) was used for comparative assays. The specificity of pertussis antibody was evaluated by carrying out direct agglutination and seroneutralization tests on 2988 serum samples at different age groups.

Results and Discussion

Figures 1 and 2 show the seroreaction of *B.pertussis* stained strains No.509 and Tohama with WHO standard antiserum. Comparison of all tested seroreactions reveal that the agglutination of the stained germs is more clear when Tohama wild strain was used. Therefor this strain was choosen for SPA preparation. The prepared antigen was used as a laboratory tool for rapid clinical diagnosis of the whooping cough in our study.

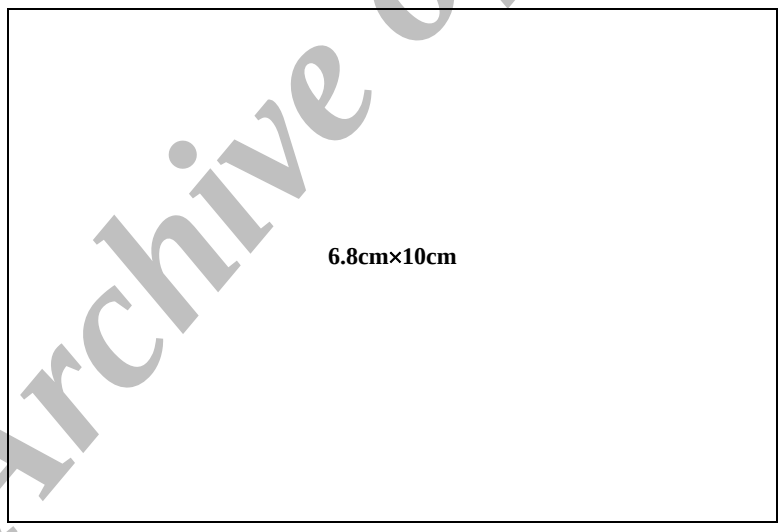

Figure 1. *Seroreaction of B.pertussis stained strain No.509 with WHO standard antiserum (BBA=Brucella buffered antigen diluent)*

Each unit of agglutinin of anti pertussis serum contains the amount of γ-globulin can neutralize 4mg of killed pertussis bacteria (minimum letal dose for white mouse

of 16-18gr body weight) (Ajjan 1992). Based on this information each drop (1/20cc) of our stained antigen contains 4mg of inactivated germ of Tohama wild strain. (Figure 2).

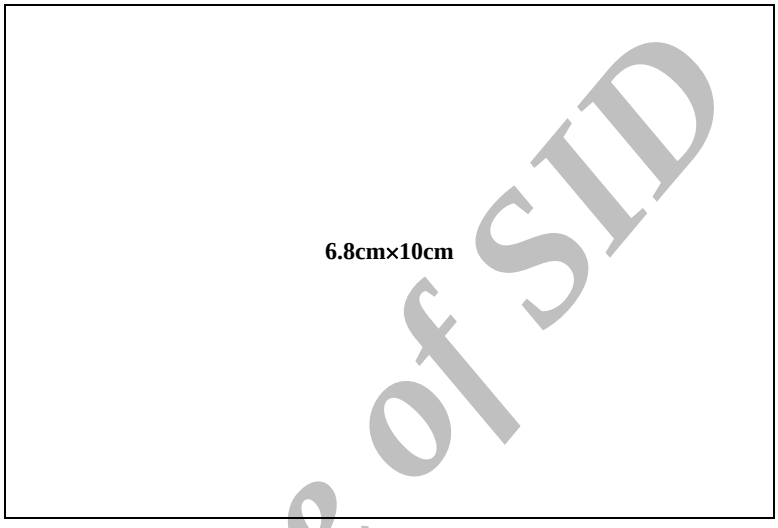

Figure 2. *Seroreaction of B.pertussis stained Tohama wild strain with WHO standard antiserum (BBA=Brucella buffered antigen diluent)*

To determine the specificity pertussis antibody, a comparative study between seroneutralization and direct agglutination (using *B.pertussis* stained Tohama strain antigen) tests on 2988 serum samples at different age groups was done (Table 1). The result indicates that the sensitivity and specificity of direct agglutination test using the stained antigen are similar to seroneutralization test. Thus the prepared SPA can provide a useful laboratory tool for rapid clinical diagnosis and evaluation of the pertussis immune response in populations.

It confirms that at least 80 units of agglutinating antibody per each ml of serum appear after five times vaccination against pertussis (Ajjan 1992), thus infection with *B.pertussis* is the only cause for generating a titer higher than this. In our study the two age groups, 51-60 and 61-70, are more sensetive to pertussis infection. Some

epidemiological studies (Cherry 1998, Keitel & Edwards 1995, Rosental *et al* 1995, Edwards *et al* 1993) suggested that after regulating vaccination against pertussis, the age group of 40-50 is more sensitive population. Because vaccination of more than 6 years old is not recommented (Keitel 1999, Nilsson *et al* 1998, Manclark & Cowell 1994), the only cause for increasing the titer would be infection of adults with the bacterium. This phenomenon is know in aged people as chronic bronchopneumonia (Hewlett 1992, Keitel & Edwards 1995, Shefer *et al* 1995).

Table 1. *Mean antibody titer of B.pertussis stained Tohama strain antigen in different age groups*

Age group	No. serum sample	SD	Stained antigen Direct agglutination	Seroneutralization
1-10	218	5.09	72,38	72,38
11-20	456	7.14	63,55	63,55
21-30	644	8.79	54,37	54,37
31-40	595	3.03	36,93	36,93
41-50	337	1.84	27/27	27/27
51-60	406	11.02	65/25	65/25
61-70	268	9.71	71/15	71/15
71-80	64	4.38	80/00	80/00

Refrences

Ajjan, N. (1992). *Vaccination. Pasteur Merieux Serums and Vaccines*. Pp: 32:132.

Alton, G.G., Jones, L.M. (1988). Techniques for the Brucellosis laboratory. *WHO and FAO Manual.*

Bemis, D.A., Burn, J.R. (1994). Bordetella pathogenesis of infections in animals. *Bordetella* Pp:201-215. Academic Press.

Cherry, J. (1998). Pertussis in adults. *Annual International Medicine* 128:64-65.

Edwards, K.M., Decker, M.D., Graham, B.S., Mezzaresta, J., Scott, J. and Hackell, J. (1993). Adult immunization with acellular pertussis vaccine. *Journal of American Medical Association* 269:53-56.

He, Q., Viljanen, M.K., Nikkari, S., Lyytikainen, R. and Mortsola, J. (1994). Outcomes of *Bordetella pertussis* infection in different age groups of an immunized population. *Journal of Infection Disease* 170:873-877.

Hewlett, E. (1992). Pertussis in adults: significance for disease transmission and immunization policy. *Journal of Medical Microbiology* 36:141-142.

Keitel, W.A. (1999). Cellular and acellular pertussis vaccines in adults. *Clinical Infectious Disease* 28:118-123.

Keitel, W.A. and Edwards, K.M. (1995). Pertussis in adolescents and adults: time to reimmunize? *Seminar of Respiratory Infection* 10:51-57.

Kwantes, W., Joynson, D.H.M. and Williams, W.O. (1983). *Bordetella pertussis* isolation in general practice:1977-79 whooping cough epidemic in West Glomorgan. *Journal of Hygiene* 90:149-158.

Manclark, C.R., Cowell, J.L. (1993). *Pertussis. Bacterial Vaccines*.Pp:69-106. Academic Press.

Mirchamsy, H. (1997). Pertussis. General topics on: Prevantion and Treatment by Vaccine and Sera (3rd edn.), Pp:179-212. Tehran University Publication. (In Persian).

Muller, A.S., Leeunenburg, J. and Pratt, D.S. (1986). Pertussis: Epidemiology and control. *Bulletin of WHO*. Pp:321.

Nilsson, L., Gruber, C., Granstorom, M., Bjorksten, B. and Kejellman, N.I. (1998). Pertussis IgE and atopie disease. *Allergy* 53:1195-1201.

Rosental, S., Brusuelas, K., Snaden, G., Sterbel, P. and Wharton, M. (1995). Pertussis infection among adults during the 1993 outbreak in Chicago. *Journal of Infectious Disease* 171:1650-1652.

Ruuskamen, O., Noel, A., Putto-Laurila, A., Peter, J., Capiau, C., Delem, A., Vandervoorde, D., Simoen, E., Teuwen, D.E., Bogaerys, H. and Andre, F.E. (1991). Development of an acellular pertussis vaccine and its administration as a booster in healthy adults. *Vaccine* 79:117-121.

Ryan, M. (1997). *Bordetella pertussis* respiratory infection in children is associated with preperential activation of type 1 Th cell. *Journal of Infectious Disease* 175:1246-1250.

Shefer, A., Nelson, M., Werner, B., baron, R. and Jackson, R. (1995). Use of safety acellular pertussis vaccine among adult hospital staff during an outbreak of pertussis. *Journal of Infectious Disease* 171:1053-1056.