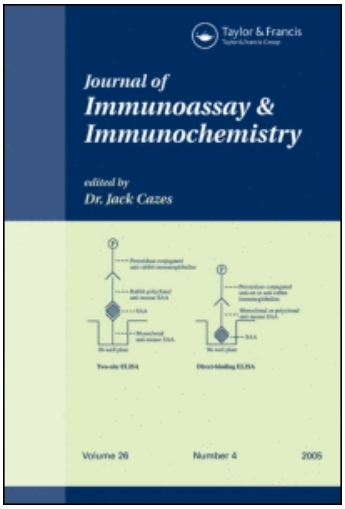

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EVALUATION OF PROTEIN-A LINKED MONOCLONAL ANTIBODY LATEX AGGLUTINATION TEST FOR DIAGNOSIS OF NUCLEAR POLYHEDROSIS VIRUS (BmNPV) OF SILKWORM BOMBYX MORI L.

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ABSTRACT

The symptomology of BmNPV infection in Bombyx mori L depends on the stages of infection. Discernible symptoms develop at later stages of infection, which leads to improper diagnosis and poor crop yield with sericulturists. In the present study development of direct and protein-A linked monoclonal antibody latex (PALMAL) agglutination test for the detection of BmNPV infection in silkworm is described. Latex beads were precoated with protein-A and then sensitised with monoclonal antibody MA-231 (125μg/ml). PALMAL test could detect $1X10^5$ nuclear polyhedra/test and is ten times more sensitive than the direct agglutination test employing purified polyclonal antibodies. No agglutination was observed in presence of B. thuringiensis, S. marcescens, N. bombycis, group-A streptococci, Staphylococcus aureus, E. coli and normal haemolymph protein indicating the specificity of the test. Fifty haemolymph samples collected from the field were evaluated by PALMAL test. Twenty one samples having infection other than BmNPV, failed to show positive agglutination. Twenty five samples having $\geq 5X10^6$ BmNPV/ml showed positive agglutination. However, 4 samples having $<5X10^6$ BmNPV/ml failed to show positive agglutination thereby indicating the limit of sensitivity of the assay.

(**KEY WORDS:** Nuclear polyhedra of BmNPV, Monoclonal antibody, protein-A linked latex agglutination test.)

INTRODUCTION

The infectious nuclear polyhedrosis virus (BmNPV) of Bombyx mori is economically important baculovirus which causes nuclear polyhedrosis disease in silkworm B. mori L. The symptoms of this disease are observed at the late stage of infection which leads to death of infected larvae and hence poor crop yield for sericulturists. In India, 20-30% crop loss is due to infection by this virus and hence a suitable diagnostic method for putting in evidence the BmNPV infection at an early stage is required. Serological technique such as latex agglutination tests for the detection of flacherie (Shimizu et al., 1983), cytoplasmic polyhedrosis (Shimizu and Arakawa, 1986) and densonucleosis (Shimizu and Tauchi 1991) viruses in silkworm have been proposed. Arakawa (1989) introduced a latex agglutination test for the diagnosis of nuclear polyhedrosis virus in silkworm. Recently, Arakawa and Shimizu (1990) developed a protein-A linked latex agglutination test for the detection of nuclear polyhedrosis virus and the test was found to be 4-5 times more sensitive than the direct latex agglutination test. These tests have employed polyclonal antibodies.

In the present study we report the development of a sensitive and specific latex agglutination test for the early detection of BmNPV at the field level employing monoclonal antibody coupled to protein-A coated latex

particles. The result obtained by this test have also been compared with polyclonal antibody based direct latex agglutination test.

MATERIALS AND METHODS

Purification of Nuclear Polyhedra

The nuclear polyhedra of BmNPV were collected from infected silkworm by homogenisation of larvae in 5 volume of water. The polyhedra were purified from the homogenate by washing four times with physiological saline (0.9% NaCl) followed by centrifugation on a 40-63%(w/w) sucrose density gradient. Polyhedra were counted, aliquoted and stored at -20^{o}C for further use.

Production of Polyclonal and Monoclonal Antibodies

The polyclonal antibodies against nuclear polyhedra of BmNPV in rabbits and murine monoclonal antibodies (MCAs) were generated as described previously by Shamim et al. (1994). Low degree of crossreactivity of polyclonal antibodies with haemolymph protein was removed by adsorption of pooled high titred immune sera with CNBr activated sepharose-4B coupled to normal haemolymph protein.

The monoclonal antibody MA-321 used in this study is of IgG1 isotype. It reacted well with different strains of nuclear polyhedra such as *B. mori*, *A. olbistriga*, *H. armigera*, *S. litura* and had no reactivity with *B. thuringiensis*, *S. marcescens*, *N. bombycis*, group-A streptococci, *Staphylococcus aureus* & *E. coli*.

Development of Direct Latex Agglutination Test Using Polyclonal Antibody

Latex polystyrene beads (0.74μm, Polysciences, U.S.A) were washed two times with distilled water and once with 0.1 M glycine buffered saline (GBS pH 8.2) by centrifugation at 9000 rpm for 5min. The latex beads (final concentration 0.4%) were incubated with 400, 200, 125 μg/ml of anti-BmNPV IgG in GBS at 37^{o}C for 2hr followed by overnight at 4^{o}C with continuous gentle end to end mixing. The sensitised beads were centrifuged at 5000rpm for 10min, washed once with GBS, containing 0.2% BSA and suspended in GBS-BSA containing 0.05% sodium azide. The sensitised beads were stored at 4^{o}C.

Protein-A Linked Monoclonal Antibody Latex (PALMAL) Agglutination Test

Polystyrene latex beads were coated with 48, 24, 12, 6 and 3 μg/ml of protein-A as described for polyclonal antibodies. Following sensitization, beads were washed in GBS for further use. To each of the above protein-A coated latex particles, four different concentrations (i.e., 400, 250, 125, and 100 μg/ml) of monoclonal antibody (MA-321) were incubated at 37^{o}C for 2hr and followed by 4^{o}C overnight with continuous end to end mixing. After washing twice with GBS containing 0.2% BSA the sensitized latex particles were finally suspended in GBS-BSA containing 0.05% sodium azide.

Assay

Nuclear polyhedra ($20X10^5/25\mu l$ PBS and at doubling dilutions) was mixed with $25\mu l$ of GBS and $50\mu l$ of sensitized latex particles on a glass slide. The slide was slowly rotated for 2-3 min, put on a flat surface and observed for visible agglutination. The test was graded as negative in the absence of visible agglutination and positive agglutination was graded as strongly positive (+++) and positive (++). The control included instead of nuclear polyhedra, $25\mu l$ of PBS, haemolymph or Nosema bombycis spores ($28X10^6$).

Detection of Nuclear Polyhedra in field samples by PALMAL Agglutination Test

Forty five larvae suspected to be infected with BmNPV and 5 with Nosema bombycis were collected from Hindupur (Andhra Pradesh), Kolar and Mysore (Karnataka). Haemolymph was obtained by homogenizing the larva in 2 ml of PBS followed by centrifugation at 200 rpm for 5min. Supernatant was tested for the presence of nuclear polyhedra by PALMAL test. In addition samples were examined microscopically and results compared with PALMAL agglutination test.

RESULTS

Using checker board analysis, a combination of $6\mu g$ protein-A/ml and $125\mu g$ MA-321/ml were found to give optimun result (Table 1). In contrast for direct latex

TABLE 1.

DETERMINATION OF OPTIMUM CONCENTRATIONS OF PROTEIN-A AND MONOCLONAL ANTIBODY FOR THE DETECTION OF NUCLEAR POLYHEDRA IN PALMAL AGGLUTINATION TEST

Protein-A conc. (μg/ml)	Concentration of monoclonal antibody (μg/ml)			
	400	250	125	100
48	+++	+++	+++	++
24	+++	+++	+++	++
12	+++	+++	+++	++
6	+++	+++	+++	-
3	++	++	-	-
Nil	-	-	-	-

Nuclear polyhedra used $5X10^5$/test.

+++ strong positive agglutination.
++ positive agglutination.
- no agglutination.

agglutination 250μg polyclonal antibody/ml gave optimum agglutination (data not shown). Using PALMAL test positive agglutination was observed with $1X10^5$ nuclear polyhedra per test. In contrast $10X10^5$ nuclear polyhedra per test were required to observe positive agglutination in a direct latex agglutination test based on polyclonal antibodies, thereby suggesting that PALMAL test is 10 times more sensitive than direct latex agglutination test (Table 2). Employing the same batch of monoclonal antibody sensitized latex particles,

TABLE 2.

THE COMPARISON OF SENSITIVITY OF PALMAL TEST VERSUS DIRECT LATEX AGGLUTINATION TEST FOR THE DETECTION OF NUCLEAR POLYHEDRA OF BmNPV

Concentration of nuclear polyhedra per test	Agglutination	
	PALMAL agglut. test	Direct latex agglut. test
$20X10^5$	+++	+++
$10X10^5$	+++	++
$5X10^5$	+++	-
$2.5X10^5$	++	-
$1X10^5$	++	-
$0.5X10^5$	-	-

Positive agglutination was observed within 2-3 min.

+++ strong positive
++ positive
- no agglutination

PALMAL test showed consistent sensitivity of the order of $1X10^5$ nuclear polyhedra when repeated five times. Moreover, three batches of sensitized latex particles prepared as per the procedure described above, were able to detect $1X10^5$ nuclear polyhedra per test.

Moreover, PALMAL test was highly specific as no agglutination was observed in presence of B. thuringiensis, S. marcescens, N. bombycis, E. coli, group-A streptococci, Staph. aureus and healthy haemolymph protein (Table 3). However, direct latex agglutination gave

TABLE 3.

THE REACTIVITY PATTERN OF PALMAL VERSUS DIRECT LATEX AGGLUTINATION TEST WITH DIFFERENT PATHOGENS OF BOMBYX MORI L

Antigen	PALMAL agglut. test	Direct Latex agglut.Test
Nuclear Polyhedra (BmNPV)	+++	+++
Bacillus thuringiensis	-	++
Serratia marcescens	-	-
Nosema bombycis	-	-
E. coli	-	-
Streptococcus-A	-	-
Staph aureus	-	-
Healthy larval haemolymph protein (10μg/ml)	-	-

+++ strong positive
++ positive
- no agglutination

positive agglutination in presence of B. thuringiensis. Fifty haemolymph samples collected from the field were evaluated by the PALMAL test. Twenty five samples having nuclear polyhedra higher than $5X10^6$/ml as counted microscopically, gave clear visible agglutination. However, 4 samples containing lesser amount of nuclear polyhedra (i.e., 10^2 - 10^4/ml) showed no agglutination. In addition, sixteen samples with bacterial and fungal contamination also showed no visible agglutination thereby confirming specificity of the assay. Five

TABLE 4.

DETECTION OF NUCLEAR POLYHEDRA IN HAEMOLYMPH SAMPLES OF BOMBYX MORI LARVAE COLLECTED FROM FIELD BY PALMAL AGGLUTINATION TEST

No. of samples	Microscopic Examination	Agglutination
25	BmNPV infection ($\geq 5X10^6$*)	+++
4	BmNPV infection ($<5X10^6$*)	-
15	Bacterial infection	-
1	Fungus infection	-
5	Nosema bombycis	-

* Number of nuclear polyhedra per ml.
Field samples (50) collected from Hindupur (A.P.), Kolar (Karnataka) and the farmer's houses from Mysore.

+++ strong positive
- no agglutination

control samples consisting of N. bombycis spore were also found to be negative with this test (Table 4).

DISCUSSION

In the present study the feasibility of utilization of protein-A linked monoclonal antibody latex agglutination test for the detection of nuclear polyhedra of BmNPV in silkworm B. mori L has been described. The assay employs MCA, MA-321 directed against polyhedrin protein of nuclear polyhedra which is highly specific and there is no cross-reactivity with other pathogens and haemolymph of healthy silkworm larva (Shamim et al, 1994). The latex particles sensitized with monoclonal

antibody failed to show any agglutination in presence of BmNPV. It may be due to (i) steric hindrance caused by the attachment of the monoclonal antibodies to the solid support at a region close to its active site, (ii) conformational changes or (iii) restriction of the intramolecular flexibility imposed by multi-site attachment to the solid support as described previously by van Erp et al, 1992. In PALMAL agglutination test latex particles are sensitized with monoclonal antibody through a protein-A bridge. This may provide easy accessibility of MCA to bind the antigen. Sensitized latex particles when stored at $4^{o}C$ were stable upto six month, the period for which the investigations were performed. The test is simple, quick, specific and 10 times sensitive than direct latex agglutination test employing polyclonal antibodies. Apart from sensitivity and specificity PALMAL test requires less amount of antibody (125μg/ml) as compared to direct agglutination test (250μg/ml). The improved sensitivity of PALMAL test may be due to firstly the use of specific monoclonal antibody and secondly the free floating antigen binding sites (Fab) of the immunoglobulin to react with antigen which has earlier been explained by Torrance (1980).

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REFERENCES

1. Shimizu, S., Ohaba, M., Kanda, K. and Aizawa, K. Latex agglutination test for the detection of flacherie virus of the silkworm, Bombyx mori L. J. Invertebr. Pathol. 1983; 42: 151-5.

2. Shimizu, S. and Arakawa, A. Latex agglutination test for the detection of the cytoplasmic polyhedrosis virus and the densonucleosis virus of the silkworm, Bombyx mori L. J. Seric. Sci. Japan. 1986; 56:169-70.

3. Shimizu, S. and Tauchi, S. Protein-A coated latex linked antiserum test for the detection of the densonucleosis virus of the silkworm, Bombyx mori L. J. Invertebr. Pathol. 1991;57: 124-5.

4. Arakawa, A. Diagnosis of nuclear polyhedrosis by latex agglutination test in the silkworm, Bombyx mori. J. Seric. Sci. Japan. 1989; 58: 257-8.

5. Arakawa, A. and Shimizu, S. Agglutination test using protein-A coated latex particles for the detection of nuclear polyhedrosis virus of silkworm, Bombyx mori (Lepidoptera: Bombycidae). Appl. Ent. Zool. 1990; 25: 519-21.

6. Shamim, M., Baig, M., Datta, R. K. and Gupta, S. K. Development of monoclonal antibody based sandwich ELISA for the detection of nuclear polyhedra of nuclear polyhedrosis virus infection in Bombyx mori L. J. Invertebr. Pathol. 1994; 63: 151-6.

7. van Erp, R., Linders, Y. E. M., van Sommeren, A.P.G. and Gribnan, T. C. J. Characterization of mono-

clonal antibodies physically adsorbed onto polystyrene latex particles. J. Immunol. Methods. 1992; 120: 191-9.

8. Torrance, L. Use of protein-A to improve sensitization of latex particles with antibodies to plant virus. Ann. Appl. Biol. 1980; 96: 45-50.