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(71) Applicant *(for all designated States except US)*: **COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH** [IN/IN]; Rafi Marg, New Delhi 110 001 (IN).

(71) Applicant and
(72) Inventor: **RANI, Bangalore, Eshwar, Amita** [IN/IN]; Central Food Technological Research Institute, Mysore, Karnataka 570 013 (IN).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **KUMARI, Chokkadi, Ganapathi, Udaya** [IN/IN]; Central Food Technological Research Institute, Mysore, Karnataka 570 013 (IN). **KARANTH, Nitturu, Gopalakrishna** [IN/IN]; Central Food Technological Research Institute, Mysore, Karnataka 570 013 (IN).

(74) Agent: **BHOLA, Ravi**; K & S Partners, 84-C, C6 Lane, Sainik Farms, Off. Central Avenue, New Delhi 110 062 (IN).

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(54) Title: A PROCESS FOR PREPARING EGG YOLK ANTIBODIES

(57) Abstract: The present invention relates to generating polyclonal antibodies from egg yolk antibodies to specific protein myosin obtained from insects Sitophilus oryzae, Tribolium castaneum, Rhizopertha dominica and Acheta domestica.

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A PROCESS FOR PREPARING EGG YOLK ANTIBODIES

TECHNICAL FIELD

The present invention relates to generating polyclonal antibodies from egg yolk antibodies to specific protein myosin obtained from insects *Sitophilus oryzae*, *Tribolium castaneum*, *Rhizopertha dominica* and *Acheta domestica*

BACKGROUND OF THE INVENTION

Insect infestation in stored grain is a universal problem and causes loss to food grain. Insect contamination results in 20-30% loss of food, both in quality and quantity. Pest infestation also reduces the quality of the produce and its marketability. Thus, there is a need to monitor insect infestation to ensure food quality. Detection of insects and insect parts in stored whole grain and milled products is a problem for the cereal and milling industry. The current methods rely on visual examination of grain insects and damaged kernels (Nicholson *et al* 1953 and Hill 1990). These methods need well-trained personnel and are time consuming besides they are not effective in detecting low level of hidden or internally developing insects, Other experimental techniques include x-ray analysis, nuclear magnetic resonance spectroscopy (Street & Bruce 1976) and chemical techniques (Ashman *et al* 1969 and Laessig *et al* 1972). However, none of these methods meet the requirement of an **"ideal assay".** An ideal assay would be specific, sensitive, inexpensive, easy to perform and adaptable to granaries and grain mills. Biochemical /immunochemical methods meet this requirement.

In recent years, techniques of producing antibodies (polyclonal and monoclonal) have been developed which make it possible to obtain homogenous, highly specific antibodies. Generally, polyclonal antibodies are used extensively in diagnostics industry. Most commonly they are raised in mammals such as rabbit, mice, rat, horse and goat. This form of antibody production has several disadvantages – large mammals are expensive to maintain, while small mammals yield small quantities of antibody. In addition there is a requirement for periodic drawing of blood from the animals (Deignan et al 2000). The amount of IgG (antibody) obtained is usually between 3-8 mg/ml of serum. The method also involves bleeding of the rabbit several times to obtain the antibodies, as the titer is highest only between the 8^{th} – 10^{th} day after 2-3 boosters. Monoclonal antibodies are produced by immunizing an animal with a protein, obtaining antibody-producing cells from the animal, and fusing the antibody-producing cells with strains of myeloma cells, i.e., tumor cells, to produce hybridomas, which are isolated

and cultured as monoclones. The monoclonal hybridomas may either be cultured in vitro or from the cells, ascitic fluid, or serum of a tumor-bearing host animal. Since each antibody-producing cell generates a single unique antibody, each monoclonal culture of hybridomas produces a homogeneous antibody. Not all of the hybridoma clones, which result from fusing myeloma cells with antibody-producing cells, are specific for the desired analyte (or ligand specific), since many of the hybridomas will make antibodies, which the inoculated animal has produced to react with other foreign substances. Even antibodies against the subject antigen will differ from clone to clone, since antibodies produced by different cells may react with different antigenic determinants of the same molecule. From each clone, therefore, it is necessary to obtain the resulting antibody and test its reactivity with the analyte and to test its specificity by determining which particular organochlorines pesticide it recognizes. Further, only certain antibodies or antisera function in specific immunoassay formats or configurations.

The present invention relates to the production of egg yolk antibodies for an insect specific protein myosin. The use of antibodies from the egg yolks of hyperimmunized hens (IgY antibody) for immunological procedures overcomes the limitations associated with the polyclonal and monoclonal antibodies, because the present method provides a continuous supply of large quantities of consistent, high titer specific and sensitive antibody which can be easily collected and stored. The manufacture and collection of hen egg antibodies is well understood in the field of clinical medicine.

United States. Pat. Nos. 4,387,272 and 4,550,019 to Polson; and Losch, U claimed production of hen egg yolk antibodies and have been used in a number of applications for passive transfer of immunity.

United States. Pat No. 4,748,018 to Stolle, et al. discloses a method of passive immunization against bacterial infection comprising a preliminary development of tolerance of IgY by repeated oral ingestion of egg yolk, followed by parenteral injection of IgY antibody to a selected bacterial antigen.

United States .Pat.no.DE19737453A1 to Fischer Mattias (1997) for the claims made in the above patent for antibodies developed for elements

United States .Pat.no.03784 to Wells ,Jack,N. (1992)discloses production of monoclonal antibodies against radioactive substituted xanthines 8- (cycloalkyl) substituted – 1,3- Dipropylxanthines and polyclonal antibodies in Dekalb laying hens.

United States. Pat.no.14518 to Mark et al (1994) for antibodies developed for peptides and proteins with weekly boosters

United States. Pat No. 5,080,895 to Tokoro discloses prevention of E. Coli diarrhea in newborn piglets by oral administration of anti-bacterial hen egg yolk antibodies.

Reference is made to Hamada, S., Infection and Immunity 59(11): 4161-4167 (1991); and Otake, S., J. Dental Research 70(3): 162-166 (1991) reproduced the results of Beck in protecting rats against dental caries by means of passive immunization with orally administered hen egg yolk antibodies against S. mutans.

Reference is made to Bartz, C. et al., J. Infectious Disease 142(3): 439-441 (1980) prevented murine rotaviral infection in mice by the oral administration of the water-soluble fraction of the eggs of immunized hens.

Reference is made to Chen and Kitto, Fd & Agr Immunology (1993) 5, 165 - 175, Species specific immunoassay for S.granaries ,S.oryzae, S. zeaminis in wheat using monoclonal antibodies in mice and polyclonal antibodies in rabbits.

Reference is made to Schatzki et al., J. Econ. Entomol (1993) 86 (5): 1584-89 ELISA for Acheta domestica and S. granaries in wheat using polyclonal rabbit antibodies.

Reference is made to Yokoyama, H., et al. Infection and Immunity 60(3): 998-1007 (1992) succeeded in passively protecting neonatal piglets from fatal enterotoxigenic E. coli infection by oral administration of a crude yolk immunoglobulin fraction from the eggs of immunized hens.

Reference is made to the animal studies of Yolken, R. et al., Pediatrics 81(2): 291-295 (1988); and Journal Clint. Immunol. 10(6): 80S-87S (1990), proposed the oral administration of antiviral IgY immunoglobulin for the prevention and treatment of enteric infections, including rotaviral infection in humans. Method and formulations for the oral administration of immunoglobulin are known (U.S. Pat. No. 4,477,432 to Hardie.

Reference is made to Amita rani et al (2001) PCT patent pending , Development of a process for egg yolk antibodies for organochlorine pesticides.

Reference is made to Amita rani et al (2001) PCT patent pending , Development of a process for egg yolk antibodies for Cyclodiene pesticides.

Reference is made to Amita rani et al (2002) PCT patent pending , Development of a process for egg yolk antibodies for organophosphate pesticides.

Reference is made to Amita rani et al (2001) PCT patent pending , Development of a

test method based on egg yolk antibodies for organochlorine pesticides. However, there are no reports on the production of egg yolk antibodies for an insect specific protein.

OBJECT OF THE INVENTION

The main object of the present invention relates to a process of producing egg yolk antibodies against insect specific protein.

Yet another embodiment of the present invention relates to a process of isolating and purifying insect specific protein from the femur muscle of the grain insects.

Another object of the present invention relates to the process of isolating antibodies from the water soluble fraction of the egg yolk

DESCRIPTION OF THE ACCOMPANYING FIGURES/DRAWINGS

Figure 1. Development of ELISA to detect staes of *S.oryzae*

Figure 2. Development of ELISA using egg yolk antibodies

Figure 3. Structure of Egg yolk antibodies and rabbit antibodies

SUMMARY OF THE INVENTION

The present invention is to develop a process to produce egg yolk antibodies which have high titer, consistent quality of antibody, easy to produce and non invasive. The present invention of egg yolk antibodies thus has very practical economical and important advantages. Firstly, the present technique gives high yield of antibody. Secondly, production of the titer of the antibody remains high for a longer period of time almost for 60 days, thereby providing a continuous supply of consistent quality of antibody. Thirdly, it is non invasive and hence there is no need to bleed the animal to recover the required antibody as is necessarily in Rabbit antibody. The egg yolk antibody thus produced is more/equally sensitive to the polyclonal/monoclonal antibodies produced using mammals.

DETAIL DESCRIPTION OF THE INVENTION

The present invention involves a process of developing egg yolk antibodies. The egg yolk antibody thus produced is more/equally sensitive to the polyclonal/monoclonal antibodies produced using mammals.

Accordingly, the main embodiment of the present invention relates to a process for the producing egg yolk antibodies against insect specific protein said method comprising the steps of:

(a) selecting suitable poultry birds,

(b) immunizing the suitable bird of step (a) by injecting insect specific purified

protein along with Freund's complete adjuvant of about 1000μg in breast muscle of the bird,

(c) repeating the immunization by injecting insect specific purified protein along with Freund's incomplete adjuvant at two, three and five weeks intervals and again after another five weeks interval, and

(d) harvesting and isolating the antibodies from the egg yolk of the birds.

Another embodiment of the present invention relates to the poultry bird in step (a) wherein the poultry bird is selected is White Leghorn.

Still another embodiment of the present invention relates to the insects wherein the insects are selected from group comprising of *Acheta domestica*, *Sitophilus oryzae*, *Rhizopertha dominica* and *Tribolium casteneum*.

One more embodiment of the present invention relates to the injected specific purified protein along with Freund's complete adjuvant of the insect in step (a) wherein said injected specific purified protein is in the range of about 450 to 500μg.

Yet another embodiment of the present invention relates to the injected specific purified protein along with Freund's incomplete adjuvant during the intervals two, three and five weeks and again after another five weeks interval periods in step (c) wherein the range of the injected purified protein is in the range of about 200 to 300 μg

Another embodiment of the present invention relates to a process of isolating and purifying the insect specific myosin protein from femur muscles of the stored grain insects said method comprising the steps of:

(a) isolating the myosin protein from the femur of the poultry bird using phosphate buffer of pH 7.5 to obtain of the protein in the range of about 1 to 7 mg/L by convention method,

(b) purifying the protein obtained in step (a) using acetonitrile in the range of about 60 to 80%, trifluoroacetic acid in the range of about 0.05 to 2.0%, and

(c) identifying the purified protein of step (c) at a wavelength of 280nm, with a absorbance unit of full scale (AUFs) of 0.08 in run time of 25 minutes and a peak at 17 minutes.

Yet another embodiment of the present invention relates to the yield of protein from insect grain *Acheta doemstica* wherein the said yield of the protein is in the range of about 1.5 to 2.0 mg/L.

Still another embodiment of the present invention relates to the yield of protein from insect grain *Acheta doemstica* wherein the said protein is about 1.76 mg/L.

Still another embodiment of the present invention relates to the yield of protein from insect grain *Stiophilus oryzae* is wherein the said protein in the range of about 5 to 7 mg/L

Still another embodiment of the present invention relates to the yield of protein from insect grain *Stiophilus oryzae* is wherein the said protein is about 5.6 mg/L

Still another embodiment of the present invention relates to the yield of protein from insect grain *Rhizopertha dominica* is in the range of about 1 to 5 mg/L

Still another embodiment of the present invention relates to the yield of protein from insect grain *Rhizopertha dominica* is about 1.6 mg/L

Still another embodiment of the present invention relates to the yield of protein from insect grain *Tribolium castaneu* is in the range of about 1 to 5mg/L

Still another embodiment of the present invention relates to the yield of protein from insect grain *Tribolium castaneu* is about 2 mg/.

Another embodiment of the present invention relates to the conventional method in step (a) wherein said conventional method is centrifugation and SDS-PAGE.

Yet another embodiment of the present invention relates to the purified protein in step (c) wherein the said purified protein from insect *Acheta domestica* is about 200 kDa and from insects *Stiophilus oryzae*, *Rhizopertha dominica* and *Tribolium castaneu* is about 45 kDa.

Another embodiment of the present invention relates the a process of isolating antibodies wherein antibodies against the specific insect protein are isolated from water-soluble fraction of the egg yolk, said process comprising of:

(a) rupturing the egg and obtaining to obtain lipid contents of the egg yolk,

(b) diluting the lipid contents of egg yolk of step (a) with water followed by addition of kappa carragenan in the range of about 50 to 75 % to obtain water soluble protein fraction antibodies (WSPF),

(c) passing the WSPF antibodies from step (b) through sodium hypophosphate in the range of about 0.5 to 2 mM,

(d) precipitating the WSPF antibodies obtained from step (c) with PEG 8000 in the range of about 10-20 %, and

(e) purifying the WSPF antibodies obtained from step (d) by affinity column chromatography

Still another embodiment of the present invention relates to the kappa carragenan in

step (b) wherein kappa carragenan is about 60%.

One more embodiement of the present invention relates to the sodium hypophosphate in step (c), wherein sodium hypophosphate is about 1mM

Yet another embodiment of the present invention relates to the PEG 8000 in step (d) wherein PEG 8000 is about 14%.

Another embodiment of the present invention relates to the yield of antibodies wherein the yield of antibodies from *Acheta domestica*, *Sitophilus oryzae*, *Rhizopertha dominica* and *Tribolium casteneum* protein is in the range of about 80-98 %.

Still another embodiment of the present invention relates to yield of antibodies wherein the yield of the antibodies from *Acheta domestica*, *Sitophilus oryzae*, *Rhizopertha dominica* and *Tribolium casteneum* protein is about 95 %.

One more embodiment of the present invention relates to the production of the antibody wherein the production of the antibody is initiated from the day 7 after immunization and continues upto 60 days.

Yet another embodiment of the present invention relates to the antibody produced wherein the antibody produced from insect *Acheta domestica* is in the range of about 5 - 15g /hen, from insect *Sitophilus oryzae* is in the range of about 3 -15g/hen, from insect *Rhizopertha dominica* is in the range of about 3 -10g /hen and from insect *Tribolium castaneum* is in the range of about 3 – 15 g /hen.

Still another embodiment of the present invention relates to the antibody produced wherein the antibody produced from insect *Acheta domestica* is in the range of about 6 - 12g /hen, from insect *Sitophilus oryzae* is in the range of about 4.3 –10 g/hen, from insect *Rhizopertha dominica* is in the range of about 4 –8 g /hen and from insect *Tribolium castaneum* is in the range of about 5 – 9 g /hen.

One more embodiment of the present invention relates to the egg yolk antibodies wherein the egg yolk antibodies are useful for analysis of insect contamination and filth in foods by ELISA (Fig.1).

Another embodiment of the present invention relates to the egg yolk antibodies produced provide the basic material for developing ELISA test methods using plate, tube, dipstick, PVP films and biosensors (Fig.2).

Still another embodiment of the present invention relates to the developed ELISA test methods wherein developed ELISA test methods the find application in the detection of insect contamination /filth in food, milled products and processed foods. (Fig.1 and

Table 3)

Table 1: Purification of antibodies from egg yolk

Sl.No.	Method	Percentage yield	Sensitivity
1	CHANGE OF pH	80	+++
2	CHLOROFORM	75	++
3	DEAE-SEPHACEL COLUMN	80	+++
4	DEXTRAN SULPHATE	40	+
5	GUMS	80	+++
6	MAGNESIUM CHLORIDE AND PHOSPHOTUNGSTIC ACID	60	++
7	POLYETHYLENE GLYCOL	75	+++
8	PATENTED METHOD	95	++++

Table 2: Comparison between present invention and Prior Arts

Sl.No.	Present Invention	Chen and Kitto	Schalzi et al
1	Antibodies against insects *Acheta domestica*, *Sitophilus oryzae*, *Rhizopertha dominica* and *Tribolium castaneum*	Antibodies against *S.granaris*	Antibodies against *S.granaries*
2	Egg yolk antibodies from hen –IgY	Antibodies from rabbit-IgG	Antibodies from rabbit-IgG
3	Antibodies purified from insects, *Acheta domestica*, *Sitophilus oryzae*, *Rhizopertha dominica* and *Tribolium castaneum*	Antigen purified from *S.granaris*	Antigen purified from *Acheta domestica*
4	Whole IgY-antibody molecule	Whole IgG-antibody molecule	Fragment of antibody –Fab
5	Indirect ELISA format	Sandwitch ELISA format	Sandwitch ELISA format
6	Extraction buffer-0.2M	Extraction Buffer – 50 mM	Extraction buffer- 150 mM

Table 3: Spike and Recovery

Number of Insect Spiking in 100 g of food	Number of insect recovery	Percentage
1	1.2	120
5	3.6	72
50	27.0	54

The following examples are given by way of illustration of the present invention and therefore should not be construed to limit the scope of the present invention.

EXAMPLES

EXAMPLE –1

Accordingly the present invention provides a process for the production of egg yolk antibodies for an insect specific protein, which comprises of periodic intra muscular immunization of the poultry birds with the insect specific protein (500 μg of the purified protein) in the breast muscle at 3-4 sites. Individual poultry (White Leghorn birds) were immunized with the immunogen (insect protein myosin). The initial immunization using 50% Freund's complete adjuvant which is an emulsifier and helps in diffusion of the antigen and made up to one mL with normal saline was followed by 250μg per bird in Freund's incomplete adjuvant, two, three and five weeks later and at five weeks interval thereafter. Antibodies were harvested from egg yolk.

EXAMPLE –2

The insect protein was obtained from the femur muscle of the field insect cricket – Acheta domestica. The protein was purified by the modified method of Chaplain and Tregear (1996) and Woods et al (1963). The femurs (10g) were separated from the field insect cricket and used to isolate the protein myosin. The femurs were ground with cold extraction phosphate buffer pH at 7.5. The extract was centrifuged at 3000xg for 5 min. The supernatant collected and stored as crude extract. Cold deionised water was added to the pellet and centrifuged at 20,000x g for 30 min. The pellet was resuspended in 0.2 M phosphate buffer + NaCl, at 4^0 for 1 hour, centrifuged at 16,000 x g for 25 min. The supenatant was collected and stored at – 80^0 C. Yield – 1.76mg/mL.

EXAMPLE –3

The insect protein was obtained from the stored grain insect Sitophilus oryzae. Whole insects were used to obtain the protein. The protein was purified by the modified method of Chaplain and Tregear (1996) and Woods et al (1963).

Whole insects (50g) were used to isolate the protein myosin. The insects were ground with cold extraction phosphate buffer pH at 7.5. The extract was centrifuged at 3000xg for 5 min. The supernatant collected and stored as crude extract. Cold deionised water was added to the pellet and centrifuged at 20,000x g for 30 min. The pellet was resuspended in 0.2 M phosphate buffer + NaCl, at 4^0 for 1 hour, centrifuged at 16,000 x g for 25 min. The supenatant was collected and stored at – 80^0 C.

Yield – 5.6 mg/mL or 112mg/20mL.

EXAMPLE –4

The insect protein was obtained from the insect Rhizopertha dominica (2g). The protein

was purified by the modified method of Chaplain and Tregear (1996) and Woods et al (1963).

Whole insects (2g) were used to isolate the protein myosin. The insects were ground with cold extraction phosphate buffer pH at 7.5. The extract was centrifuged at 3000xg for 5 min. The supernatant collected and stored as crude extract. Cold deionised water was added to the pellet and centrifuged at 20,000x g for 30 min. The pellet was resuspended in 0.2 M phosphate buffer + NaCl, at 4 ^{0} for 1 hour, centrifuged at 16,000 x g for 25 min. The supenatant was collected and stored at – 80 ^{0} C.

Yield – 1.6mg /mL or 20.8mg/13mL

EXAMPLE –5

The insect protein was obtained from the femur muscle of the stored grain pest – Tribolium castaneum. The protein was purified by the modified method of Chaplain and Tregear (1996) and Woods et al

(1963).

Whole insects (2g) were used to isolate the protein myosin. The insects were ground with cold extraction phosphate buffer pH at 7.5. The extract was centrifuged at 3000xg for 5 min. The supernatant collected and stored as crude extract. Cold deionised water was added to the pellet and centrifuged at 20,000x g for 30 min. The pellet was resuspended in 0.2 M phosphate buffer + NaCl, at 4 ^{0} for 1 hour, centrifuged at 16,000 x g for 25 min. The supenatant was collected and stored at – 80 ^{0} C. Yield – 2 mg/m

EXAMPLE –6

The insect specific protein myosin purified from cricket Acheta domestica was checked for its purity using SDS – PAGE. A resolving gel of 8%, stacking gel – 5% at 100 V was found to be most suitable. The purified protein from Acheta domestica was analysed for its purity using Poly Acrylamide Gel Electrophoresis. 30% acrylamide solution was poured into the glass plates assembled upright and a clean Teflon comb inserted. The gel was allowed to solidify at room temperature. Tris glycine electrophoresis buffer (pH 8.3) was added to the reservoirs. The gel was mounted in the electrophoresis apparatus.

50ug of sample and 50ug standard molecular weight marker (myosin 20 KD) were loaded on the gel in a predetermined order. A power supply of 100V for 6-7 hrs was supplied to the electrophoresis apparatus. After the completion of the run, the gel was transferred into a staining trough and stained with Coomassie brilliant blue for 4 hr at

room temperature on a rocker. The gel was then destained for 5-8hrs at room temperature in a destaining solution consisting of Methanol and glacial acetic acid. The destaining solution was changed 3 or 4 times. The bands seen were two distinct bands were detected in the standard. One major band corresponded to myosin heavy chain (MHC) (200KD) and the other could be paramyosin subunit (41 KD) .

EXAMPLE –7

The insect specific protein myosin purified from stored grain insect Sitophilus oryzae was checked for its purity using SDS – PAGE. A resolving gel of 8%, stacking gel – 5% at 100 V was found to be most suitable.

The purified protein from Sitophilus oryzae was analysed for its purity using Poly Acrylamide Gel Electrophoresis. 30% acrylamide solution was poured into the glass plates assembled upright and a clean Teflon comb inserted. The gel was allowed to solidify at room temperature. Tris glycine electrophoresis buffer (PH 8.3) was added to the reservoirs. The gel was mounted in the electrophoresis apparatus.

50ug of sample and 50ug standard molecular weight marker (myosin 20 KD) were loaded on the gel in a predetermined order. A power supply of 100V for 6-7 hrs was supplied to the electrophoresis apparatus. After the completion of the run, the gel was transferred into a staining trough and stained with Coomassie brilliant blue for 4 hr at room temperature on a rocker. The gel was then destained for 5-8hrs at room temperature in a destaining solution consisting Methanol and 8 mL of glacial acetic acid. The destaining solution changed 3 or 4 times. The bands seen were two distinct bands were detected in the standard. One major band corresponded to myosin heavy chain (MHC) (200KD) and other band could be paramyosin subunit (41 KD).

EXAMPLE –8

The insect specific protein myosin purified from the insect Rhizopertha Dominica was checked for its purity using SDS – PAGE. A resolving gel of 8%, stacking gel – 5% at 100 V was found to be most suitable.

The purified protein from Rhizopertha dominica was analysed for its purity using Poly Acrylamide Gel Electrophoresis. 30% acrylamide solution was poured into the glass plates assembled upright and a clean Teflon comb inserted. The gel was allowed to solidified at room temperature. Tris glycine electrophoresis buffer (PH 8.3) was added to the reservoirs. The gel was mounted in the electrophoresis apparatus. 50ug of sample and 50ug standard molecular weight marker(myosin 20 KD) were loaded on the gel in a

predetermined order. A power supply of 100V for 6-7 hrs was supplied to the electrophoresis apparatus. After the completion of the run, the gel was transferred into a staining trough and stained with Coomassie brilliant blue for 4 hr at room temperature on a rocker. The gel was then destained for 5-8hrs at room temperature in a destaining solution consisting methanol and glacial acetic acid. The destaining solution changed 3 or 4 times. The bands seen were two distinct bands were detected in the standard. One major band corresponded to myosin heavy chanin (MHC) (200KD) the other band could be paramyosin subunit (41 KD).

EXAMPLE –9

The insect specific protein myosin purified from the insect Triobolium castaneum was checked for its purity using SDS – PAGE. A resolving gel of 8%, stacking gel – 5% at 100 V was found to be most suitable.

The purified protein from Tribolium castaneum was analysed for its purity using Poly Acrylamide Gel Electrophoresis. 30% acrylamide solution was poured into the glass plates assembled upright and a clean Teflon comb inserted. The gel was allowed to solidify at room temperature. Tris glycine electrophoresis buffer (PH 8.3) was added to the reservoirs. The gel was mounted in the electrophoresis apparatus. 50ug of sample and 50ug standard molecular weight marker(myosin 20 KD) were loaded on the gel in a predetermined order. A power supply of 100V for 6-7 hrs was supplied to the electrophoresis apparatus. After the completion of the run, the gel was transferred into a staining trough and stained with Coomassie brilliant blue for 4 hr at room temperature on a rocker. The gel was then destained for 5-8hrs at room temperature in a destaining solution consisting of methanol and glacial acetic acid. The destaining solution changed 3 or 4 times. The bands seen were two distinct bands were detected in the standard. One major band corresponded to myosin heavy chain (MHC) (200KD). The other band could be paramyosin subunit (41 KD).

EXAMPLE –10

The insect specific protein myosin purified from cricket Acheta domestica was checked for its purity using HPLC with a mobile phase comprising of 70 % acetonitrile, and 0.1% trifluroacetic acid at a wavelength of 280 nm, flow rate 1ml/min and Aufs of 0.08 and run time of 75 mins. The peaks were not resolved well

EXAMPLE –11

The insect specific protein myosin purified from cricket Acheta domestica was checked

for its purity using HPLC with the mobile phase of 70 % acetonitrile, 0.1% trifluroacetic acid at a wavelength of 230 nm, flow rate 1ml/min and Aufs of 0.08 and run time of 45 mins. The peaks were not resolved well

EXAMPLE –12

The insect specific protein myosin purified from cricket Acheta domestica was checked for its purity using HPLC with the mobile phase of 70 % acetonitrile, 0.1% trifluroacetic acid at a wavelength of 230 nm, flow rate 1ml/min and Aufs of 0.08 and run time of 25 mins.

Three major peaks were detected at 2.96, 9.65 and 17.0 minutes. The peak at 2.96 was the solvent peak, peak at 9.65 was the paramyosin and the protein (Myosin) peak was detected at 17th minute.

EXAMPLE –13

The insect specific protein myosin purified from stored grain pest – Sitophilus oryzae was checked for its purity using HPLC with the mobile phase of 70 % acetonitrile, 0.1% trifluroacetic acid at a wavelength of 280 nm, flow rate 1ml/min and Aufs of 0.08 and run time of 75 mins. The peaks were not resolved well

EXAMPLE –14

The insect specific protein myosin purified from stored grain pest – Sitophilus oryzae was checked for its purity using HPLC with the mobile phase of 70 % acetonitrile, 0.1% trifluroacetic acid at a wavelength of 230 nm, flow rate 1ml/min and Aufs of 0.08 and run time of 45 mins. The peaks were not resolved well

EXAMPLE –15

The insect specific protein myosin purified from stored grain pest – Sitophilus oryzae was checked for its purity using HPLC with the mobile phase of 70 % acetonitrile, 0.1% trifluroacetic acid at a wavelength of 230 nm, flow rate 1ml/min and Aufs of 0.08 and run time of 25 mins.

Three major peaks were detected at 2.96, 9.65 and 17.0 minute. The peak at 2.96 was the solvent peak, 9.65 was the paramyosin peak and the protein (Myosin) peak was detected at 17th minute.

EXAMPLE –16

The insect specific protein myosin purified from stored grain pest – Rhizopertha dominica as checked for its purity using HPLC with the mobile phase of 70 % acetonitrile, 0.1% trifluroacetic acid at a wavelength of 280 nm, flow rate 1ml/min and

Aufs of 0.08 and run time of 75 mins. The peaks were not resolved well

EXAMPLE –17

The insect specific protein myosin purified from stored grain pest – Rhizopertha dominica was checked for its purity using HPLC with the mobile phase of 70 % acetonitrile, 0.1% trifluroacetic acid at a wavelength of 230 nm, flow rate 1ml/min and Aufs of 0.08 and run time of 45 mins. The peaks were not resolved well

EXAMPLE –18

The insect specific protein myosin purified from stored grain pest – Rhizopertha dominica was checked for its purity using HPLC with the mobile phase of 70 % acetonitrile, 0.1% trifluroacetic acid at a wavelength of 230 nm, flow rate 1ml/min and Aufs of 0.08 and run time of 25 mins.

Three major peaks were detected at 2.96, 9.65 and 17.0 minute. The peak at 2.96 was the solvent peak, 9.65 was the paramyosin peak and the protein (myosin) peak was detected at 17th minute.

EXAMPLE –19

The insect specific protein myosin purified from stored grain pest – Tribolium castaneum as checked for its purity using HPLC with the mobile phase of 70 % acetonitrile, 0.1% trifluroacetic acid at a wavelength of 280 nm, flow rate 1ml/min and Aufs of 0.08 and run time of 75 mins. The peaks were not resolved well

EXAMPLE –20

The insect specific protein myosin purified from stored grain pest – Tribolium castaneum was checked for its purity using HPLC with the mobile phase of 70 % acetonitrile, 0.1% trifluroacetic acid at a wavelength of 230 nm, flow rate 1ml/min and Aufs of 0.08 and run time of 45 mins. The peaks were not resolved well

EXAMPLE –21

The insect specific protein myosin purified from stored grain pest – Tribolium castaneum was checked for its purity using HPLC with the mobile phase of 70 % acetonitrile, 0.1% trifluroacetic acid at a wavelength of 230 nm, flow rate 1ml/min and Aufs of 0.08 and run time of 25 mins.

Three major peaks were detected at 2.96, 9.65 and 17.0 minute. The peak at 2.96 was the solvent peak, 9.65 was the paramyosin peak and the protein (Myosin) peak was detected at 17th minute.

EXAMPLE –22

The purified protein from cricket femur muscle was injected into white Leghorn bird's poultry. The initial immunization using 50% Freund's complete adjuvant (500 μg of the purified protein) was followed by 250μg per bird in Freund's incomplete adjuvant, two, three and five weeks later and at five weeks interval thereafter.

EXAMPLE –23

The purified protein from the stored grain pest – Sitophilus oryzae was injected into white Leghorn bird's poultry. The initial immunization using 50% Freund's complete adjuvant (500 μg of the purified protein) was followed by 250μg per bird in Freund's incomplete adjuvant, two, three and five weeks later and at five weeks interval thereafter.

EXAMPLE –24

The purified protein from the stored grain pest – Rhizopertha Dominica was injected into white Leghorn bird's poultry. The initial immunization using 50% Freund's complete adjuvant (500 μg of the purified protein) was followed by 250μg per bird in Freund's incomplete adjuvant, two, three and five weeks later and at five weeks interval thereafter.

EXAMPLE –25

The purified protein from stored grain pest – Tribolium castaneum was injected into white Leghorn bird's poultry. The initial immunization using 50% Freund's complete adjuvant (500 μg of the purified protein) was followed by 250μg per bird in Freund's incomplete adjuvant, two, three and five weeks later and at five weeks interval thereafter.

EXAMPLE –26

The production of the antibody (Acheta domestica, Sitophilus Oryzae, Rhizopertha dominica and Tribolium castaneum) started on day 7 after immunization and was continuous for 60 days.

EXAMPLE –27

The eggs from the birds immunised with protein myosin obtained from Acheta domestica were collected from the 7^{th} day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was then ruptured and the contents transferred into a measuring cylinder through a funnel. For every 10 ml of

yolk, 100ml of Tris buffer saline, pH 7.4 was added. The precipitate formed was removed by centrifugation at 2000g for 30 minutes. To the supernatant the precipitating solution of magnesium chloride (3 M) and phosphotungstic acid (4.8%) were added and centrifuged again. The pellet was discarded. To the supernatant now called the watersoluble protein fraction, 12%. polyethylene glycol (PEG 6000) was added and incubated for 10 minutes and then centrifuged. The antibody precipitated out. 10 ml of 10mM phosphate buffer was added and the precipitate dissolved. The antibody solution is then cooled to 0^0C and 10 ml of pre cooled ethanol added. The solution was centrifuged at 4^0C and the sediment was dissolved in 10 mM phosphate buffer and dialysed against 3 changes of phosphate buffer for 24 h at 4 ^{0}C.Yield - 60%.

The eggs from the birds immunised with protein myosin obtained from Sitophilus Oryzae were collected from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was then ruptured and the contents transferred into a measuring cylinder through a funnel. For every 10 ml of yolk, 100ml of Tris buffer saline, pH 7.4 was added. The precipitate formed was removed by centrifugation at 2000g for 30 minutes. To the supernatant the precipitating solution of magnesium chloride (3M) and phosphotungstic acid (4.8%) were added and centrifuged again. The pellet was discarded. To the supernatant now called the watersoluble protein fraction, 12% polyethylene glycol (PEG 6000) was added and incubated for 10 minutes and then centrifuged. The antibody precipitated out. 10 ml of 10mM phosphate buffer was added and the precipitate dissolved. The antibody solution is then cooled to 0^0C and 10 ml of pre cooled ethanol added. The solution was centrifuged at 4^0C and the sediment was dissolved in 10 mM phosphate buffer and dialysed against 3 changes of phosphate buffer for 24 h at 4 ^{0}C.Yield - 60%.

The eggs from the birds immunised with protein myosin obtained from Rhizopertha dominica were collected from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was then ruptured and the contents transferred into a measuring cylinder through a funnel. For every 10 ml of

yolk, 100ml of Tris buffer saline, pH 7.4 was added. The precipitate formed was removed by centrifugation at 2000g for 30 minutes. To the supernatant the precipitating solution of magnesium chloride (3M) and phosphotungstic acid (4.8%) were added and centrifuged again. The pellet was discarded. To the supernatant now called the watersoluble protein fraction, 12% polyethylene glycol (PEG 6000) was added and incubated for 10 minutes and then centrifuged. The antibody precipitated out. 10 ml of 10mM phosphate buffer was added and the precipitate dissolved. The antibody solution is then cooled to 0^0C and 10 ml of pre cooled ethanol added. The solution was centrifuged at 4^0C and the sediment was dissolved in 10 mM phosphate buffer anddialysed against 3 changes of phosphate buffer for 24 h at 4 ^{0}C.Yield - 60%.

The eggs from the birds immunised with protein myosin obtained from Tribolium castaneum were collected from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was then ruptured and the contents transferred into a measuring cylinder through a funnel. For every 10 ml of yolk, 100ml of Tris buffer saline, pH 7.4 was added. The precipitate formed was removed by centrifugation, at 2000 g for 30 minutes. To the supernatant the precipitating solution of magnesium chloride (3M) and phosphotungstic acid (4.8%) were added and centrifuged again. The pellet was discarded. To the supernatant now called the waters soluble protein fraction, 12% polyethylene glycol was added and incubated for 10 minutes and then centrifuged. The antibody precipitated out. 10 ml of 10mM phosphate buffer was added and the precipitate dissolved. The antibody solution is then cooled to 0^0C and 10 ml of pre cooled ethanol added. The solution was centrifuged at 4^0C and the sediment was dissolved in 10 mM phosphate buffer and dialysed against 3 changes of phosphate buffer for 24 h at 4 ^{0}C.Yield - 60%.

EXAMPLE –28

The eggs from the birds immunised with protein myosin obtained from Acheta domestica were collected from the 7th day after the first booster was used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was then ruptured and transferred the contents into a measuring cylinder through a funnel. For every 10 ml of yolk, 100ml of

Tris buffer saline, pH 7.4 was added. The precipitate formed was removed by centrifugation at 2000g. To the supernatant the precipitating solution of magnesium chloride (3M) and phosphotungstic acid (4.8%) was added and centrifuged again. The pellet was discarded. To the supernatant now called the water soluble protein fraction 12% polyethylene glycol was added and incubated for 10 minutes and then centrifuged. The antibody precipitated out. 10 ml of 10mM phosphate buffer were added and the precipitate dissolved. The antibody solution was then cooled to 0^0C and 10 ml of pre cooled ethanol added. The solution was centrifuged at 4^0C and the sediment was dissolved in 10 mM phosphate buffer and dialysed against 3 changes of phosphate buffer for 24 h at 4 ^{0}C. To increase the yield of antibody, the lipid from the egg yolk was precipitated out twice using the precipitating solution of phosphotungstic acid and magnesium chloride to the supernatant and centrifuged. Yield – 75%.

The eggs from the birds immunised with protein myosin obtained from Sitophilus oryzae were collected from the 7th day after the first booster were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was then ruptured and transferred the contents into a measuring cylinder through a funnel. For every 10 ml of yolk, 100ml of Tris buffer saline, pH 7.4 was added. The precipitate formed was removed by centrifugation, at 2000g for 30 minutes. To the supernatant the precipitating solution of magnesium chloride (3M) and phosphotungstic acid (4.8%) was added and centrifuged again. The pellet was discarded. To the supernatant now called the water soluble protein fraction 12% polyethylene glycol (PEG 6000) was added and incubated for 10 minutes and then centrifuged. The antibody precipitated out. 10 ml of 10mM phosphate buffer were added and the precipitate dissolved. The antibody solution was then cooled to 0^0C and 10 ml of pre cooled ethanol added. The solution was centrifuged at 4^0C and the sediment was dissolved in 10 mM phosphate buffer and dialysed against 3 changes of phosphate buffer for 24 h at 4 ^{0}C. To increase the yield of antibody, the lipid from the egg yolk was precipitated out twice using the precipitating solution of phosphotungstic acid and magnesium chloride to the supernatant and centrifuged. Yield – 75%.

The eggs from the birds immunised with protein myosin obtained from *Rhizopertha dominica* were collected from the 7th day after the first booster were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell

without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was then ruptured and transferred the contents into a measuring cylinder through a funnel. For every 10 ml of yolk, 100ml of Tris buffer saline, pH 7.4 was added. The precipitate formed was removed by centrifugation at 2000g for 30 minutes. To the supernatant the precipitating solution of magnesium chloride (3M) and phosphotungstic acid (4.8%) was added and centrifuged again. The pellet was discarded. To the supernatant now called the water soluble protein fraction 12% polyethylene glycol was added and incubated for 10 minutes and then centrifuged. The antibody precipitated out. 10 ml of 10mM phosphate buffer were added and the precipitate dissolved. The antibody solution was then cooled to 0^0C and 10 ml of pre cooled ethanol added. The solution was centrifuged at 4^0C and the sediment was dissolved in 10 mM phosphate buffer and dialysed against 3 changes of phosphate buffer for 24 h at 4 ^{0}C. To increase the yield of antibody, the lipid from the egg yolk was precipitated out twice using the precipitating solution of phosphotungstic acid and magnesium chloride to the supernatant and centrifuged.Yield – 75%.

The eggs from the birds immunised with protein myosin obtained from Tribolium castaneum were collected from the 7th day after the first booster were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was then ruptured and transferred the contents into a measuring cylinder through a funnel. For every 10 ml of yolk, 100ml of Tris buffer saline, pH 7.4 was added. The precipitate formed was removed by centrifugation at 2000g for 30 minutes. To the supernatant the precipitating solution of magnesium chloride (3M) and phosphotungstic acid (4.8%) was added and centrifuged again. The pellet was discarded. To the supernatant now called the water soluble protein fraction 12% polyethylene glycol (PEG 6000) was added and incubated for 10 minutes and then centrifuged. The antibody precipitated out. 10 ml of 10mM phosphate buffer were added and the precipitate dissolved. The antibody solution was then cooled to 0^0C and 10 ml of pre cooled ethanol added. The solution was centrifuged at 4^0C and the sediment was dissolved in 10 mM phosphate buffer and dialysed against 3 changes of phosphate buffer for 24 h at 4 ^{0}C. To increase the yield of antibody, the lipid from the egg yolk was precipitated out twice using the precipitating solution of phosphotungstic acid and magnesium chloride to the supernatant and centrifuged. Yield – 75%.

EXAMPLE –29

The eggs from the birds immunised with protein myosin obtained from Acheta domestica were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. For every 10 ml of yolk, 100ml of Tris buffer saline, pH 7.4 was added. The precipitate formed was removed by centrifugation at 2000g for 30 minutes. To the supernatant the precipitating solution of magnesium chloride (3M) and phosphotungstic acid (4.8%) was added and centrifuged again. The pellet discarded and to the supernatant now called the water soluble protein fraction 12% polyethylene glycol (PEG 6000) was added and incubated for 10 minutes and then centrifuged. The antibody precipitates out. 10 ml of 10mM phosphate buffer is added and the precipitate dissolved. The antibody solution is then cooled to 0^0C and 10 ml of pre cooled ethanol added. The solution is centrifuged at 4^0C and the sediment is dissolved in 10 mM phosphate buffer and dialysed against phosphate buffer for 24 h at 4 ^{0}C. The lipid from the egg yolk was precipitated out twice using the precipitating solution of phosphotungstic acid and magnesium chloride to the supernatant and centrifuged.The pH of the water soluble protein fraction obtained after the removal of the lipids was checked and adjusted to pH 5.0 in order to further precipitate out the antibodies.Yield – 80 –90%.

The eggs from the birds immunised with protein myosin obtained from Sitophilus oryzae were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. For every 10 ml of yolk, 100ml of Tris buffer saline, pH 7.4 was added. The precipitate formed was removed by centrifugation at 2000g for 30 minutes. To the supernatant the precipitating solution of magnesium chloride (3M) and phosphotungstic acid (4.8%) was added and centrifuged again. The pellet discarded and to the supernatant now called the water soluble protein fraction 12% polyethylene glycol (PEG 6000) was added and incubated for 10 minutes and then centrifuged. The antibody precipitates out. 10 ml of 10mM phosphate buffer is added

and the precipitate dissolved. The antibody solution is then cooled to 0^0C and 10 ml of pre cooled ethanol added. The solution is centrifuged at 4^0C and the sediment is dissolved in 10 mM phosphate buffer and dialysed against phosphate buffer for 24 h at 4 ^{0}C. The lipid from the egg yolk was precipitated out twice using the precipitating solution of phosphotungstic acid (4.8%)and magnesium chloride (3M) to the supernatant and centrifuged.The pH of the water soluble protein fraction obtained after the removal of the lipids was checked and adjusted to pH 5.0 in order to further precipitate out the antibodies.Yield – 80 –90%.

The eggs from the birds immunised with protein myosin obtained from Rhizopertha dominica were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. For every 10 ml of yolk, 100ml of Tris buffer saline, pH 7.4 was added. The precipitate formed was removed by centrifugation at 2000g for 30. To the supernatant the precipitating solution of magnesium chloride (3M) and phosphotungstic acid (4.8%) was added and centrifuged again. The pellet discarded and to the supernatant now called the water soluble protein fraction 12% polyethylene glycol (PEG 6000) was added and incubated for 10 minutes and then centrifuged. The antibody precipitates out. 10 ml of 10mM phosphate buffer is added and the precipitate dissolved. The antibody solution is then cooled to 0^0C and 10 ml of pre cooled ethanol added. The solution is centrifuged at 4^0C and the sediment is dissolved in 10 mM phosphate buffer and dialysed against phosphate buffer for 24 h at 4 ^{0}C. The lipid from the egg yolk was precipitated out twice using the precipitating solution of phosphotungstic acid and magnesium chloride to the supernatant and centrifuged. The pH of the water soluble protein fraction obtained after the removal of the lipids was checked and adjusted to pH 5.0 in order to further precipitate out the antibodies. Yield – 80 –90%.

The eggs from the birds immunised with protein myosin obtained from Tribolium castaneum were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred

into a measuring cylinder through a funnel. For every 10 ml of yolk, 100ml of Tris buffer saline, pH 7.4 was added. The precipitate formed was removed by centrifugation at 2000g for 30 minutes. To the supernatant the precipitating solution of magnesium chloride (3M) and phosphotungstic acid (4.8%) was added and centrifuged again. The pellet discarded and to the supernatant now called the water soluble protein fraction 12% polyethylene glycol was added and incubated for 10 minutes and then centrifuged. The antibody precipitates out. 10 ml of 10mM phosphate buffer is added and the precipitate dissolved. The antibody solution is then cooled to 0^0C and 10 ml of pre cooled ethanol added. The solution is centrifuged at 4^0C and the sediment is dissolved in 10 mM phosphate buffer and dialysed against phosphate buffer for 24 h at 4 ^{0}C. The lipid from the egg yolk was precipitated out twice using the precipitating solution of phosphotungstic acid and magnesium chloride to the supernatant and centrifuged. The pH of the water soluble protein fraction obtained after the removal of the lipids was checked and adjusted to pH 5.0 in order to further precipitate out the antibodies.Yield – 80 –90%.

EXAMPLE –30

The eggs from the birds immunised with protein myosin obtained from Acheta domestica were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The eggyolk was carefully removed from the eggshell without rupturing the yolk membrane.The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was separated and diluted with distilled water and pH adjusted to 7.4. The pH of the water-soluble protein fraction (WSPF) obtained after centrifugation was adjusted to 5.0. 14% (w/v) polyethylene glycol 8000 was added to the WSPF and incubated at room temperature and the precipitate collected after centrifugation. The precipitate was dissolved in 10 ml of 10 mM phosphate buffer and pre-cooled ethanol added and centrifuged. The sediment thus obtained was dissolved in phosphate buffer and dialysed against phosphate buffer for 24 h at 4 ^{0}C.Yield –75%.

The eggs from the birds immunised with protein myosin obtained from Sitophilus oryzae were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The eggyolk was carefully removed from the eggshell without rupturing the yolk membrane.The entire albumin adhering to the

membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was separated and diluted with distilled water and pH adjusted to 7.4. The pH of the water-soluble protein fraction (WSPF) obtained after centrifugation was adjusted to 5.0. 14% (w/v) polyethylene glycol 8000 was added to the WSPF and incubated at room temperature and the precipitate collected after centrifugation. The precipitate was dissolved in 10 ml of 10 mM phosphate buffer and pre-cooled ethanol added and centrifuged. The sediment thus obtained was dissolved in phosphate buffer and dialysed against phosphate buffer for 24 h at 4 ^{0}C.Yield –75%.

The eggs from the birds immunised with protein myosin obtained from Rhizopertha dominica were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The eggyolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was separated and diluted with distilled water and pH adjusted to 7.4. The pH of the water-soluble protein fraction (WSPF) obtained after centrifugation was adjusted to 5.0. 14% (w/v) polyethylene glycol 8000 was added to the WSPF and incubated at room temperature and the precipitate collected after centrifugation. The precipitate was dissolved in 10 ml of 10 mM phosphate buffer and pre-cooled ethanol added and centrifuged. The sediment thus obtained was dissolved in phosphate buffer and dialysed against phosphate buffer for 24 h at 4 ^{0}C.Yield –75%.

The eggs from the birds immunised with protein myosin obtained from Tribolium castaneum were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The eggyolk was carefully removed from the eggshell without rupturing the yolk membrane.The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was separated and diluted with distilled water and pH adjusted to 7.4. The pH of the water-soluble protein fraction (WSPF) obtained after centrifugation was adjusted to 5.0. 14% (w/v) polyethylene glycol 8000 was added to the WSPF and incubated at room temperature and the precipitate collected after centrifugation. The precipitate was dissolved in 10 ml of 10 mM phosphate buffer and pre-cooled ethanol added and centrifuged. The sediment thus

obtained was dissolved in phosphate buffer and dialysed against phosphate buffer for 24 h at 4 ^{0}C.Yield –75%.

EXAMPLE –31

The eggs from the birds immunised with protein myosin obtained from Acheta domestica were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered and kappa carrageenan (60%) added. The WSPF obtained after centrifugation was passed through whatmann filter paper and 1mM Sodium hypophosphate added and filtered again. Antibody purity was 75-80 %.

The eggs from the birds immunised with protein myosin obtained from Sitophilus oryzae were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered and kappa carrageenan (60%) added. The WSPF obtained after centrifugation was passed through whatmann filter paper and 1mM Sodium hypophosphate added and filtered again. Antibody purity was 75-80 %.

The eggs from the birds immunised with protein myosin obtained from Rhizopertha dominica were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered and kappa carrageenan (60%) added. The WSPF obtained after centrifugation was passed through whatmann filter paper and 1mM Sodium hypophosphate added and filtered again. Antibody purity was 75-80 %.

The eggs from the birds immunised with protein myosin obtained from Tribolium castaneum were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the

eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered and kappa carrageenan (60%) added. The WSPF obtained after centrifugation was passed through whatmann filter paper and 1mM Sodium hypophosphate added and filtered again. Antibody purity was 75-80 %.

EXAMPLE- 32

The eggs from the birds immunised with protein myosin obtained from Acheta domestica were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered and kappa carrageenan added. The WSPF obtained after centrifugation was passed through Whatmann filter paper and 1mM Sodium hypophosphate added and filtered again. The WSPF obtained was passed through a DEAE – Sephacel column and 20 mM phosphate buffer pH 8.0passed through it along with the WSPF. The column was washed and the antibody eluted out with 0.2M phosphate buffer at pH 8.0. 15% (w/v) of sodium sulphate (anhydrous) was added at 20 ^{0}C and centrifuged. The precipitate thus obtained was diluted in 100mM phosphate buffer, centrifuged and subsequent precipitate was made up in 10 mM phosphate buffer and dialysed against 10 mM phosphate buffer pH 8.0, then filtered through 0.45 μm membrane. Antibody purity – 80 %.

The eggs from the birds immunised with protein myosin obtained from Sitophilus oryzae were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered and kappa carrageenan added. The WSPF obtained after centrifugation was passed through whatmann filter paper and 1mM Sodium hypophosphate added and filtered again. The WSPF obtained was passed through a DEAE – Sephacel column and 20 mM phosphate buffer pH 8.0passed through it along with the WSPF. The column

was washed and the antibody eluted out with 0.2M phosphate buffer at pH 8.0. 15% (w/v) of sodium sulphate (anhydrous) was added at 20 ^{0}C and centrifuged. The precipitate thus obtained was diluted in 100mM phosphate buffer, centrifuged and subsequent precipitate was made up in 10 mM phosphate buffer and dialysed against 10 mM phosphate buffer pH 8.0, then filtered through 0.45 μm membrane. Antibody purity – 80 %.

The eggs from the birds immunised with protein myosin obtained from Rhizopertha dominica were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered and kappa carrageenan added. The WSPF obtained after centrifugation was passed through whatmann filter paper and 1mM Sodium hypophosphate added and filtered again. The WSPF obtained was passed through a DEAE – Sephacel column and 20 mM phosphate buffer pH 8.0passed through it along with the WSPF. The column was washed and the antibody eluted out with 0.2M phosphate buffer at pH 8.0. 15% (w/v) of sodium sulphate (anhydrous) was added at 20 ^{0}C and centrifuged. The precipitate thus obtained was diluted in 100mM phosphate buffer, centrifuged and subsequent precipitate was made up in 10 mM phosphate buffer and dialyzed against 10 mM phosphate buffer pH 8.0, then filtered through 0.45 μm membrane. Antibody purity – 80 %.

The eggs from the birds immunised with protein myosin obtained from Tribolium castaneum were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered and kappa carrageenan added. The WSPF obtained after centrifugation was passed through whatmann filter paper and 1mM Sodium hypophosphate added and filtered again. The WSPF obtained was passed through a DEAE – Sephacel column and 20 mM phosphate buffer pH 8.0passed through it along with the WSPF. The column was washed and the antibody eluted out with 0.2M phosphate buffer at pH 8.0. 15%

(w/v) of sodium sulphate (anhydrous) was added at 20 ^{0}C and centrifuged. The precipitate thus obtained was diluted in 100mM phosphate buffer, centrifuged and subsequent precipitate was made up in 10 mM phosphate buffer and dialysed against 10 mM phosphate buffer pH 8.0, then filtered through 0.45 µm membrane. Antibody purity – 80 %.

EXAMPLE –33

The eggs from the birds immunised with protein myosin obtained from Acheta domestica were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel.

One volume of egg yolk was diluted with 4 volumes of 0.1 M phosphate buffered saline and the lipid precipitated using 1 volume of chloroform in 4 volume of PBS. The aqueous layer obtained after centrifugation was treated with 14% (w/w) of polyethylene glycol 8000 and the precipitate containing the antibodies was reconstituted in PBS containing azide and glycerol. Antibody purity - 75%.

The eggs from the birds immunised with protein myosin obtained from Sitophilus oryzae were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel.

One volume of egg yolk was diluted with 4 volumes of 0.1 M phosphate buffered saline and the lipid precipitated using 1 volume of chloroform in 4 volume of PBS. The aqueous layer obtained after centrifugation was treated with 14% (w/w) of polyethylene glycol 8000 and the precipitate containing the antibodies was reconstituted in PBS containing azide and glycerol. Antibody purity – 75%.

The eggs from the birds immunised with protein myosin obtained from Rhizopertha dominica were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred

into a measuring cylinder through a funnel.

One volume of egg yolk was diluted with 4 volumes of 0.1 M phosphate buffered saline and the lipid precipitated using 1 volume of chloroform in 4 volume of PBS. The aqueous layer obtained after centrifugation was treated with 14% (w/w) of polyethylene glycol 8000 and the precipitate containing the antibodies was reconstituted in PBS containing azide and glycerol. Antibody purity – 75%.

The eggs from the birds immunised with protein myosin obtained from Tribolium castaneum were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel.

One volume of egg yolk was diluted with 4 volumes of 0.1 M phosphate buffered saline and the lipid precipitated using 1 volume of chloroform in 4 volume of PBS. The aqueous layer obtained after centrifugation was treated with 14% (w/w) of polyethylene glycol 8000 and the precipitate containing the antibodies was reconstituted in PBS containing azide and glycerol. Antibody purity – 75%.

EXAMPLE –34

The eggs from the birds immunised with protein myosin obtained from Acheta domestica were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered and kappa carrageenan (60%) added. The WSPF obtained after centrifugation was passed through whatmann filter paper and 1mM Sodium hypophosphate added and filtered again. The WSPF obtained was treated with 14% (w/v) PEG 8000 and the antibody precipitated out.

Antibody purity – 90 –95%

The eggs from the birds immunised with protein myosin obtained from Sitophilus oryzae were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the

membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered and kappa carrageenan (60%)added. The WSPF obtained after centrifugation was passed through whatmann filter paper and 1mM Sodium hypophosphate added and filtered again. The WSPF obtained was treated with 14% (w/v) PEG 8000 and the antibody precipitated out.

Antibody purity – 90 –95%

The eggs from the birds immunised with protein myosin obtained from Rhizopertha dominica were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered and kappa carrageenan (60%) added. The WSPF obtained after centrifugation was passed through whatmann filter paper and 1mM Sodium hypophosphate added and filtered again. The WSPF obtained was treated with 14% (w/v) PEG 8000 and the antibody precipitated out.

Antibody purity – 90 –95%

The eggs from the birds immunised with protein myosin obtained from Tribolium castneum were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered and kappa carrageenan (60%) added. The WSPF obtained after centrifugation was passed through whatmann filter paper and 1mM Sodium hypophosphate added and filtered again. The WSPF obtained was treated with 14% (w/v) PEG 8000 and the antibody precipitated out.

Antibody purity – 90 –95%

EXAMPLE –35

The eggs from the birds immunised with protein myosin obtained from Acheta domestica were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the

eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered and kappa carrageenan (60%) added. The WSPF obtained after centrifugation was passed through whatmann filter paper and 1mM Sodium hypophosphate added and filtered again. The WSPF obtained was treated with 14% (w/v) PEG 8000 and the antibody precipitated out. The purified antibody obtained was further purified using ligand specific affinity column. The affinity column were prepared as follows: The activation of sepharose gel (4B) was done by taking 50ml of Sepharose (4B) gel ,washed with distilled water 3 times (100 ml each time).To 50 ml of the gel, 50 ml of distilled water and 2M Sodium carbonate (1:1:1 ratio) was added. The contents were vortexed on a magnetic stirrer. 10 ml of Cyanogen bromide was added, kept in ice bath for 5 minutes with constant stirring. Washed the gel with ice-cold distilled water 4 times (100 ml each time). Washed with 0.01 M HCl 4 times (100ml each time). Added 0.1 M Sodium carbonate, containing 0.5 M of Sodium chloride (100 ml) and use for coupling reaction. It is important that all steps should be carried out under a fume hood. Cyanogen bromide (CNBr) reagent should be handled using gloves and no mouth pipetting. The activated gel thus obtained was conjugated to the Protein or IgG as follows. Estimated the amount of protein content. All steps to be carried out in cold conditions. 5ml of the activated gel at 25mg of protein (IgG/protein) in 5 ml of 0.1 M Sodium bicarbonate, containing 0.5 M Sodium chloride (pH-9). Shake gently for 10- 12 hours. Add 0.25 ml of Ethanolamine to the reactants and shake gently for 2 hours. Pack the gel in a glass or a plastic column and wash with 10 bed volumes (50×10) of 0.1 M Sodium bicarbonate, containing 0.5 M Sodium chloride (pH-9), followed by 10 mM PBS (pH-7.4). Store the gel- coupled protein in 10mM PBS, containing 0.02% Sodium Azide at 4° C. The protocol for purification of the antibody using the column was as follows: Matrix (bed volume –500 l). Wash with 3 bed volumes of the binding buffer. 500ul of the sample, diluted 1:1 with the binding buffer is loaded. The outflow was collected. The column was washed with 10 ml of the binding buffer. Aliquots of 1 ml each were collected and absorbance was read at 280 nm. The column was eluted with 10 ml of Lysine- HCl buffer (pH-2.8). Aliquots of 1 ml each were collected. Absorbance was read at 280 nm. The antibodies thus obtained had better sensitivity.

The eggs from the birds immunised with protein myosin obtained Sitophilus Oryzae

were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered and kappa carrageenan added. The WSPF obtained after centrifugation was passed through whatmann filter paper and 1mM Sodium hypophosphate added and filtered again. The WSPF obtained was treated with 14% (w/v) PEG 8000 and the antibody precipitated out. The purified antibody obtained was further purified using ligand specific affinity column. The affinity column were prepared as follows: The activation of sepharose gel (4B) was done by taking 50ml of Sepharose (4B) gel ,washed with distilled water 3 times (100 ml each time).To 50 ml of the gel, 50 ml of distilled water and 2M Sodium carbonate (1:1:1 ratio) was added. The contents were vortexed on a magnetic stirrer. 10 ml of Cyanogen bromide was added, kept in ice bath for 5 minutes with constant stirring. Washed the gel with ice-cold distilled water 4 times (100 ml each time). Washed with 0.01 M HCl 4 times (100ml each time). Added 0.1 M Sodium carbonate, containing 0.5 M of Sodium chloride (100 ml) and use for coupling reaction. It is important that all steps should be carried out under a fume hood. CNBr reagent should be handled using gloves and no mouth pipetting. The activated gel thus obtained was conjugated to the Protein or IgG as follows. All steps were carried out in cold conditions. 5ml of the activated gel at 25mg of protein (IgG/protein) in 5 ml of 0.1 M Sodium bicarbonate, containing 0.5 M Sodium chloride (pH-9). Shake gently for 10- 12 hours. Add 0.25 ml of ethanolamine to the reactants and shake gently for 2 hours. Pack the gel in a glass or a plastic column and wash with 10 bed volumes (50×10) of 0.1 M Sodium bicarbonate, containing 0.5 M Sodium chloride (pH-9), followed by 10 mM PBS (pH-7.4). Store the gel- coupled protein in 10mM PBS, containing 0.02% Sodium Azide at 4° C. The protocol for purification of the antibody using the column was as follows: Matrix (bed volume –500 l). Wash with 3 bed volumes of the binding buffer. 500ul of the sample, diluted 1:1 with the binding buffer is loaded. The outflow was collected. The column was washed with 10 ml of the binding buffer. Aliquots of 1 ml each were collected and absorbance was read at 280 nm. The column was eluted with 10 ml of Lysine- HCl buffer (pH-2.8). Aliquots of 1 ml each were collected. Absorbance was read at 280 nm. The antibodies thus obtained had

90 – 95 % sensitivity.

The eggs from the birds immunised with protein myosin obtained Rhizopertha dominica were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered and kappa carrageenan (60%) added. The WSPF obtained after centrifugation was passed through whatmann filter paper and 1mM Sodium hypophosphate added and filtered again. The WSPF obtained was treated with 14% (w/v) PEG 8000 and the antibody precipitated out. The purified antibody obtained was further purified using ligand specific affinity column. The affinity column were prepared as follows: The activation of sepharose gel (4B) was done by taking 50ml of Sepharose (4B) gel, washed with distilled water 3 times (100 ml each time). To 50 ml of the gel, distilled water and 2M Sodium carbonate (1:1:1 ratio) was added. The contents were vortexed on a magnetic stirrer. 10 ml of Cyanogen bromide was added, kept in ice bath for 5 minutes with constant stirring. Washed the gel with ice-cold distilled water 4 times (100 ml each time). Washed with 0.01 M HCl 4 times (100ml each time). Added 0.1 M Sodium carbonate, containing 0.5 M of Sodium chloride (100 ml) and use for coupling reaction. It is important that all steps should be carried out under a fume hood. CNBr reagent should be handled using gloves and no mouth pipetting. The activated gel thus obtained was conjugated to the Protein or IgG as follows. Estimated the amount of protein content. All steps to be carried out in cold conditions. 5ml of the activated gel at 25mg of protein (IgG/protein) in 5 ml of 0.1 M Sodium bicarbonate, containing 0.5 M Sodium chloride (pH-9). Shake gently for 10- 12 hours. Add 0.25 ml of Ethanolamine to the reactants and shake gently for 2 hours. Pack the gel in a glass or a plastic column and wash with 10 bed volumes (50×10) of 0.1 M Sodium bicarbonate, containing 0.5 M Sodium chloride (pH-9), followed by 10 mM PBS (pH-7.4). Store the gel- coupled protein in 10mM PBS, containing 0.02% Sodium Azide at 4° C. The protocol for purification of the antibody using the column was as follows: Matrix (bed volume –500 l). Wash with 3 bed volumes of the binding buffer. 500ul of the sample, diluted 1:1 with the binding buffer is loaded. The outflow was collected. The column was washed with 10 ml of the binding buffer. Aliquots of 1 ml each were

collected and absorbance was read at 280 nm. The column was eluted with 10 ml of Lysine- HCl buffer (pH-2.8). Aliquots of 1 ml each were collected. Absorbance was read at 280 nm. The antibodies thus obtained had better sensitivity. 90 –95% binding.

The eggs from the birds immunised with protein myosin obtained Tribolium castaneum were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered and kappa carrageenan (60%) added. The WSPF obtained after centrifugation was passed through whatmann filter paper and 1mM Sodium hypophosphate added and filtered again. The WSPF obtained was treated with 14% (w/v) PEG 8000 and the antibody precipitated out. The purified antibody obtained was further purified using ligand specific affinity column. The affinity column were prepared as follows: The activation of sepharose gel (4B) was done by taking 50ml of Sepharose (4B) gel, washed with distilled water 3 times (100 ml each time). To 50 ml of the gel, 50 ml of distilled water and 2M Sodium carbonate (1:1:1 ratio) was added. The contents were Vortexed on a magnetic stirrer. 10 ml of Cyanogen bromide was added, kept in ice bath for 5 minutes with constant stirring. Washed the gel with ice-cold distilled water 4 times (100 ml each time). Washed with 0.01 M HCl 4 times (100ml each time). Added 0.1 M Sodium carbonate, containing 0.5 M of Sodium chloride (100 ml) and use for coupling reaction. It is important that all steps should be carried out under a fume hood. CNBr reagent should be handled using gloves and no mouth pipetting. The activated gel thus obtained was conjugated to the Protein or IgG as follows. Estimated the amount of protein content. All steps to be carried out in cold conditions. 5ml of the activated gel at 25mg of protein (IgG/protein) in 5 ml of 0.1 M Sodium bicarbonate, containing 0.5 M Sodium chloride (pH-9). Shake gently for 10- 12 hours. Add 0.25 ml of Ethanolamine to the reactants and shake gently for 2 hours. Pack the gel in a glass or a plastic column and wash with 10 bed volumes (50×10) of 0.1 M Sodium bicarbonate, containing 0.5 M Sodium chloride (pH-9), followed by 10 mM PBS (pH-7.4). Store the gel- coupled protein in 10mM PBS, containing 0.02% Sodium Azide at 4° C. The protocol for purification of the antibody using the column was as follows: Matrix (bed volume –500 l). Wash with 3 bed volumes of the binding buffer. 500ul of the sample, diluted 1:1 with

the binding buffer is loaded. The outflow was collected. The column was washed with 10 ml of the binding buffer. Aliquots of 1 ml each were collected and absorbance was read at 280 nm. The column was eluted with 10 ml of Lysine- HCl buffer (pH-2.8). Aliquots of 1 ml each were collected. Absorbance was read at 280 nm. The antibodies thus obtained had better sensitivity. 90 – 95 % binding.

EXAMPLE –36

The eggs from the birds immunised with protein myosin obtained from Acheta domestica were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered and kappa carrageenan (60%) added. The WSPF obtained after centrifugation was passed through whatmann filter paper and 1mM Sodium hypophosphate added and filtered again. The WSPF obtained was treated with 14% (w/v) PEG 8000 and the antibody precipitated out. The purified antibody was passed through mercaptopyridine sepharose column purchased locally. The column was equilibrated with 2 bed volumes of binding buffer.1ml of the yolk extract was added (after it had been passed through a 0.45 m filter) along with 1 ml of the binding buffer. The binding buffer was passed at a rate of 5ml/minute. Passing the elution buffer eluted the binding buffer. The eluted solution was collected in 2ml fractions. Passing the cleaning buffer at a rate of 5ml/minute then cleaned the column. The absorbance was read at 280 nm of the 2ml fractions was taken. The fraction giving the peak absorbance was used as the purified IgG fraction. The sensitivity of the antibody did not improve.

The eggs from the birds immunised with protein myosin obtained Sitophilus Oryzae were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered and kappa carrageenan added(60%) . The WSPF obtained after centrifugation was passed through whatmann filter paper and 1mM Sodium hypophosphate added and filtered again. The WSPF obtained was treated with 14% (w/v) PEG 8000 and the

antibody precipitated out. The purified antibody was passed through mercaptopyridine sepharose column purchased locally. The column was equilibrated with 2 bed volumes of binding buffer.1ml of the yolk extract was added (after it had been passed through a 0.45 m filter) along with 1 ml of the binding buffer. The binding buffer was passed at a rate of 5ml/minute. Passing the elution buffer eluted the binding buffer. The eluted solution was collected in 2ml fractions. Passing the cleaning buffer at a rate of 5ml/minute then cleaned the column. The absorbance was read at 280 nm of the 2ml fractions was taken. The fraction giving the peak absorbance was used as the purified IgG fraction. The sensitivity of the antibody did not improve.

The eggs from the birds immunised with protein myosin obtained Rhizopertha dominica were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered and kappa carrageenan added (60%). The WSPF obtained after centrifugation was passed through whatmann filter paper and 1mM Sodium hypophosphate added and filtered again. The WSPF obtained was treated with 14% (w/v) PEG 8000 and the antibody precipitated out. The purified antibody was passed through mercaptopyridine sepharose column purchased locally. The column was equilibrated with 2 bed volumes of binding buffer.1ml of the yolk extract was added (after it had been passed through a 0.45 m filter) along with 1 ml of the binding buffer. The binding buffer was passed at a rate of 5ml/minute. Passing the elution buffer eluted the binding buffer. The eluted solution was collected in 2ml fractions. Passing the cleaning buffer at a rate of 5ml/minute then cleaned the column. The absorbance was read at 280 nm of the 2ml fractions was taken. The fraction giving the peak absorbance was used as the purified IgG fraction. The sensitivity of the antibody did not improve.

The eggs from the birds immunised with protein myosin obtained Tribolium castaneum were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered

and kappa carrageenan added (60%). The WSPF obtained after centrifugation was passed through whatmann filter paper and 1mM Sodium hypophosphate added and filtered again. The WSPF obtained was treated with 14% (w/v) PEG 8000 and the antibody precipitated out. The purified antibody was passed through mercaptopyridine sepharose column purchased locally. The column was equilibrated with 2 bed volumes of binding buffer.1ml of the yolk extract was added (after it had been passed through a 0.45 m filter) along with 1 ml of the binding buffer. The binding buffer was passed at a rate of 5ml/minute. The binding buffer was eluted by passing the elution buffer. The eluted solution was collected in 2ml fractions. Passing the cleaning buffer at a rate of 5ml/minute then cleaned the column. The absorbance was read at 280 nm of the 2ml fractions was taken. The fraction giving the peak absorbance was used as the purified IgG fraction. The sensitivity of the antibody did not improve.

EXAMPLE –37

The eggs were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. The egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered and kappa carrageenan added (60%). The WSPF obtained after centrifugation was passed through whatmann filter paper and 1mM Sodium hypophosphate added and filtered again. The WSPF obtained was treated with 14% (w/v) PEG 8000 and the antibody precipitated out. The precipitated antibody was used to see the titer by two different methods. The antibody titer was found by reading the absorbance of the purified serum at 280 nm. The Antibody titer ranged from 3 – 12 mg/mL and was 7 mg /mL on average.

EXAMPLE –38

The eggs were collected and from the 7th day after the first booster the eggs were used to purify the antibodies from the yolk. The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. The egg yolk was diluted with water and filtered and kappa carrageenan added (60%). The WSPF obtained after centrifugation was passed through whatmann filter paper and 1mM Sodium hypophosphate added and

filtered again. The WSPF obtained was treated with 14% (w/v) PEG 8000 and the antibody precipitated out. Protein estimation by Bradford method using BSA as the standard. Coomassie Brilliant blue G – 250 was dissolved in 95% ethanol and mixed with twice the volume of 85% Orthophosphoric acid and made the volume to 1000mL with water. This reagent is used to estimate the antibody titer using BSA as a standard and read the absorbance at 595nm. It can be stored at 4^0 C for almost a month.

The titer of the antibody (*Acheta domestica*) produced was 6 - 12g /hen. The titer of the antibody (*Sitophilus oryzae*) produced was 4.3 -10g/hen. The titer of the antibody (*Rhizopertha dominica*) produced was 4 - 8g /hen. The titer of the antibody (*Tribolium castaneum*) produced was 5 - 9g /hen.

ADVANTAGES

The main advantages of the present invention are

1. Antibodies kits not available commercially.
2. High yield/titer of the antibody
3. Consistent quality of the antibody
4. Sensitivity of the assay equal/better than rabbit antibodies
5. Non-invasive
6. Good affinity to the analyte

We claim:

1. A process for the producing egg yolk antibodies against insect specific protein said method comprising the steps of:
   (a) selecting suitable poultry birds,
   (b) immunizing the suitable bird of step (a) by injecting insect specific purified protein along with Freund's complete adjuvant of about 1000µg in breast muscle of the bird,
   (c) repeating the immunization by injecting insect specific purified protein along with Freund's incomplete adjuvant at two, three and five weeks intervals and again after another five weeks interval, and
   (d) harvesting and isolating the antibodies from the egg yolk of the birds.
2. A process as claimed in claim 1, wherein the poultry bird in step (a) selected is White Leghorn.
3. A process as claimed in claim 1, wherein the insects are selected from group comprising of *Acheta domestica*, *Sitophilus oryzae*, *Rhizopertha dominica* and *Tribolium casteneum*.
4. A process as claimed in claim 1, wherein injected specific purified protein along with Freund's complete adjuvant of the insect in step (a) is in the range of about 450 to 500µg.
5. A process as claimed in claim 1, wherein the injected specific purified protein along with Freund's incomplete adjuvant during the intervals two, three and five weeks and again after another five weeks interval periods in step (c) is in the range of about 200 to 300 µg.
6. A process as claimed in claim 1, wherein step (b) the insect specific myosin protein is isolated and purified from femur muscles of the stored grain insects said method comprising the steps of:
   (a) isolating the myosin protein from the femur of the stored grain insects using phosphate buffer of pH 7.5 to obtain of the protein by convention method, and
   (b) purifying the protein obtained in step (a) using acetonitrile in the range of about 60 to 80%, trifluoroacetic acid in the range of about 0.05 to 2.0%, and
   (c) identifying the purified protein of step (c) at a wavelength of 280nm,

with a absorbance unit of full scale (AUFs) of 0.08 in run time of 25 minutes and a peak at 17 minutes.

7. A process as claimed in claim 6, wherein yield of protein from grain insect *Acheta doemstica* is in the range of about 1.5 to 2.0 mg/L.

8. A process as claimed in claim 7, wherein yield of protein from grain insect *Acheta doemstica* is about 1.76 mg/L.

9. A process as claimed in claim 6, wherein yield of protein from grain insect *Stiophilus oryzae* is in the range of about 5 to 7 mg/L

10. A process as claimed in claim 9, wherein yield of protein from grain insect *Stiophilus oryzae* is about 5.6 mg/L

11. A process as claimed in claim 6, wherein yield of protein from grain insect *Rhizopertha dominica* is in the range of about 1 to 5 mg/L

12. A process as claimed in claim 11, wherein yield of protein from grain insect *Rhizopertha dominica* is about 1.6 mg/L

13. A process as claimed in claim 6, wherein yield of protein from grain insect *Tribolium castaneu* is in the range of about 1 to 5mg/L

14. A process as claimed in claim 13, wherein yield of protein from grain insect *Tribolium castaneu* is in the range of about 2 mg/.

15. A process as claimed in claim 6, wherein conventional method in step (a) is centrifugation and SDS-PAGE.

16. A process as claimed in claim 6, wherein purified protein in step (c) from grain insect *Acheta domestica* is about 200 kDa and from grain insects *Stiophilus oryzae*, *Rhizopertha dominica* and *Tribolium castaneu* is about 45 kDa.

17. A process as claimed in claim 1, wherein antibodies against the specific grain insect protein are isolated from water-soluble fraction of the egg yolk, said process comprising of:

(a) rupturing the egg and obtaining to obtain lipid contents of the egg yolk,

(b) diluting the lipid contents of egg yolk of step (a) with water followed by addition of kappa carragenan in the range of about 50 to 75 % to obtain water soluble protein fraction antibodies (WSPF),

(c) passing the WSPF antibodies from step (b) through sodium hypophosphate in the range of about 0.5 to 2 mM,

(d) precipitating the WSPF antibodies obtained from step (c) with PEG 8000 in the range of about 10-20 %, and

(e) purifying the WSPF antibodies obtained from step (d) by affinity column chromatography

18. A process as claimed in claim 17, wherein kappa carragenan in step (b) is about 60%.
19. A process as claimed in claim 17, wherein sodium hypophosphate is about 1mM
20. A process as claimed in claim 17, wherein PEG 8000 in step (d) is about 14%.
21. A process as claimed in claim 17, wherein yield of antibodies from grain insects *Acheta domestica*, *Sitophilus oryzae*, *Rhizopertha dominica* and *Tribolium casteneum* protein is in the range of about 80-98 %.
22. A process as claimed in claim 17, wherein yield of antibodies from grain insects *Acheta domestica*, *Sitophilus oryzae*, *Rhizopertha dominica* and *Tribolium casteneum* protein is in the range of about 95 %.
23. A process as claimed in claim 21, wherein the production of the antibody is initiated from the day 7 after immunization and continues upto 60 days.
24. A process as claimed in claims 1 and 17,wherein antibody produced from grain insect *Acheta domestica* is in the range of about 5 - 15g /hen, from insect *Sitophilus oryzae* is in the range of about 3 -15g/hen, from insect *Rhizopertha dominica* is in the range of about 3 -10g /hen and from insect *Tribolium castaneum* is in the range of about 3 – 15 g /hen.
25. A process as claimed in claim 24, wherein antibody produced from insect *Acheta domestica* is in the range of about 6 - 12g /hen, from insect *Sitophilus oryzae* is in the range of about 4.3 –10 g/hen, from insect *Rhizopertha dominica* is in the range of about 4 –8 g /hen and from insect *Tribolium castaneum* is in the range of about 5 – 9 g /hen.
26. A process as claimed in claims 1 and 17, wherein the egg yolk antibodies are useful for analysis of insect contamination and filth in foods by ELISA (Fig.1).
27. A process as claimed in claims 1 and 17, wherein the egg yolk antibodies produced provide the basic material for developing ELISA test methods using plate, tube, dipstick, PVP films and biosensors (Fig.2).
28. A process as claimed in claims 25 and 26, wherein the developed ELISA test methods find application in the detection of insect contamination /filth in food, milled products and processed foods. (Fig.1 and Table 3)

AMENDED CLAIMS

[(received by the International Bureau on 18 January 2005 (18.01.05); original claims 1-28 replaced by new claims 1-28 (4 pages)]

+ STATEMENT

1. A process for the producing egg yolk antibodies against insect protein said method comprising the steps of:
   (a) selecting suitable poultry birds,
   (b) immunizing the suitable bird of step (a) by injecting insect specific purified protein along with Freund's complete adjuvant of about 1000μg in breast muscle of the bird,
   (c) repeating the immunization by injecting insect specific purified protein along with Freund's incomplete adjuvant at two, three and five weeks intervals and again after another five weeks interval, and
   (d) harvesting and isolating the antibodies from the egg yolk of the birds.
2. A process as claimed in claim 1, wherein the poultry bird in step (a) selected is White Leghorn.
3. A process as claimed in claim 1, wherein the insects are selected from group comprising of *Acheta domestica*, *Sitophilus oryzae*, *Rhizopertha dominica* and *Tribolium casteneum*.
4. A process as claimed in claim 1, wherein injected purified protein along adjuvant of the insect in step (a) is in the range of about 450 to 500μg.
5. A process as claimed in claim 1, wherein the injected purified protein during the intervals two, three and five weeks and again after another five weeks interval periods in step (c) is in the range of about 200 to 300 μg.
6. A process as claimed in claim 1, wherein step (b) the insect specific myosin protein is isolated and purified from femur muscles of the stored grain insects said method comprising the steps of:
   (a) isolating the myosin protein from the femur of the stored grain insects using phosphate buffer of pH 7.5 to obtain of the protein by convention method, and
   (b) purifying the protein obtained in step (a) using acetonitrile in the range of about 60 to 80%, trifluoroacetic acid in the range of about 0.05 to 2.0%, and

(c) identifying the purified protein of step (c) at a wavelength of 280nm, with a absorbance unit of full scale (AUFs) of 0.08 in run time of 25 minutes and a peak at 17 minutes.

7. A process as claimed in claim 6, wherein yield of protein from grain insect *Acheta domestica* is in the range of about 1.5 to 2.0 mg/L.
8. A process as claimed in claim 7, wherein yield of protein from grain insect *Acheta domestica* is about 1.76 mg/L.
9. A process as claimed in claim 6, wherein yield of protein from grain insect *Stiophilus oryzae* is in the range of about 5 to 7 mg/L
10. A process as claimed in claim 9, wherein yield of protein from grain insect *Stiophilus oryzae* is about 5.6 mg/L
11. A process as claimed in claim 6, wherein yield of protein from grain insect *Rhizopertha dominica* is in the range of about 1 to 5 mg/L
12. A process as claimed in claim 11, wherein yield of protein from grain insect *Rhizopertha dominica* is about 1.6 mg/L
13. A process as claimed in claim 6, wherein yield of protein from grain insect *Tribolium castaneu* is in the range of about 1 to 5mg/L
14. A process as claimed in claim 13, wherein yield of protein from grain insect *Tribolium castaneu* is in the range of about 2 mg/.
15. A process as claimed in claim 6, wherein conventional method in step (a) is centrifugation and SDS-PAGE.
16. A process as claimed in claim 6, wherein purified protein in step (c) from grain insect *Acheta domestica* is about 200 kDa and from grain insects *Stiophilus oryzae*, *Rhizopertha dominica* and *Tribolium castaneu* is about 45 kDa.
17. A process as claimed in claim 1, wherein antibodies against the specific grain insect protein are isolated from water-soluble fraction of the egg yolk, said process comprising of:

(a) rupturing the egg and obtaining to obtain lipid contents of the egg yolk,

(b) diluting the lipid contents of egg yolk of step (a) with water followed by addition of kappa carragenan in the range of about 50 to 75 % to obtain water soluble protein fraction antibodies (WSPF),

(c) passing the WSPF antibodies from step (b) through sodium hypophosphate in the range of about 0.5 to 2 mM,

(d) precipitating the WSPF antibodies obtained from step (c) with PEG 8000 in the range of about 10-20 %, and

(e) purifying the WSPF antibodies obtained from step (d) by affinity column chromatography

18. A process as claimed in claim 17, wherein kappa carragenan in step (b) is about 60%.
19. A process as claimed in claim 17, wherein sodium hypophosphate is about 1mM
20. A process as claimed in claim 17, wherein PEG 8000 in step (d) is about 14%.
21. A process as claimed in claim 17, wherein yield of antibodies from grain insects *Acheta domestica*, *Sitophilus oryzae*, *Rhizopertha dominica* and *Tribolium casteneum* protein is in the range of about 80-98 %.
22. A process as claimed in claim 17, wherein yield of antibodies from grain insects *Acheta domestica*, *Sitophilus oryzae*, *Rhizopertha dominica* and *Tribolium casteneum* protein is in the range of about 95 %.
23. A process as claimed in claim 21, wherein the production of the antibody is initiated from the day 7 after immunization and continues upto 60 days.
24. A process as claimed in claims 1 and 17,wherein antibody produced from grain insect *Acheta domestica* is in the range of about 5 - 15g /hen, from insect *Sitophilus oryzae* is in the range of about 3 -15g/hen, from insect *Rhizopertha dominica* is in the range of about 3 -10g /hen and from insect *Tribolium castaneum* is in the range of about 3 – 15 g /hen.
25. A process as claimed in claim 24, wherein antibody produced from insect *Acheta domestica* is in the range of about 6 - 12g /hen, from insect *Sitophilus oryzae* is in the range of about 4.3 –10 g/hen, from insect *Rhizopertha dominica* is in the range of about 4 –8 g /hen and from insect *Tribolium castaneum* is in the range of about 5 – 9 g /hen.
26. A process as claimed in claims 1 and 17, wherein the egg yolk antibodies are useful for analysis of insect contamination and filth in foods by ELISA (Fig.1).

27. A process as claimed in claims 1 and 17, wherein the egg yolk antibodies produced provide the basic material for developing ELISA test methods using plate, tube, dipstick, PVP films and biosensors (Fig.2).
28. A process as claimed in claims 25 and 26, wherein the developed ELISA test methods find application in the detection of insect contamination /filth in food, milled products and processed foods. (Fig.1 and Table 3)

+ STATEMENT

In the citation D1 antibodies have been raised against reptile venom, whereas in present invention antibodies raised against the stored grain insect, which means chemistry of the two antibodies are entirely different. The citation D2 discloses antibodies raised against Lepidopterans insects whereas the present invention the antibodies have been raised against Coleopteran insects. The citation D3 discloses an obvious and most widely used adjuvant which is usually used in such kind of studies and hence is not obvious. The antibodies raised in the citation D4 are from rabbit and thus chemically different from the antibodies raised in the present study. The antibodies raised in the citation D5 is against the pesticides and not against stored grain pests, hence they are chemically and physiologically different in their actions. The inventors respectfully submit that the chemistry of the antibodies raised in the present invention is different from the prior arts. Since the chemistry of the antibodies is different therefore the person skilled in the art cannot envisage from the prior arts.

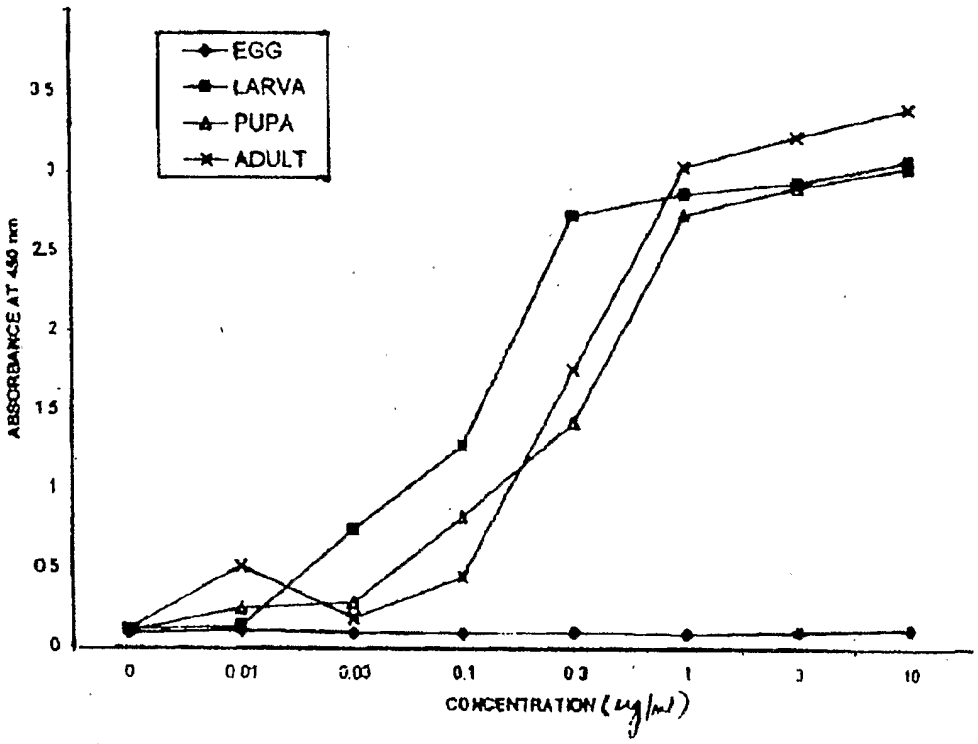
EGG
LARVA
PUPA
ADULT
ABSORBANCE AT 450 nm
0
0.5
1
1.5
2
2.5
3
3.5
0
0.01
0.03
0.1
0.3
1
3
10
CONCENTRATION (ug/ml)

Figure 1

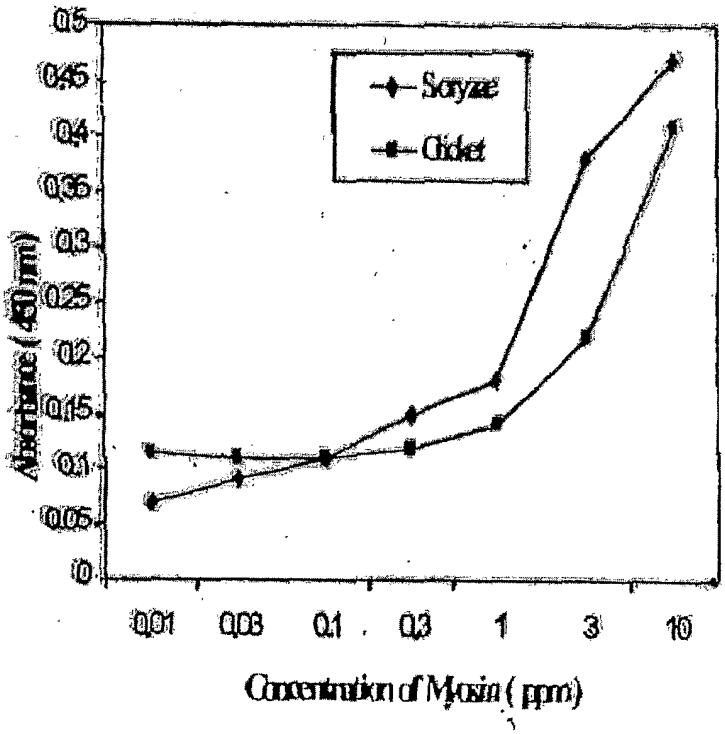
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Absorbance (450 nm)
Soryza
Oidet
0.01
0.03
0.1
0.3
1
3
10
Concentration of Mycin (ppm)

Figure 2

CH – CARBOHYDRATE MOIETY
HR – HINGE REGION

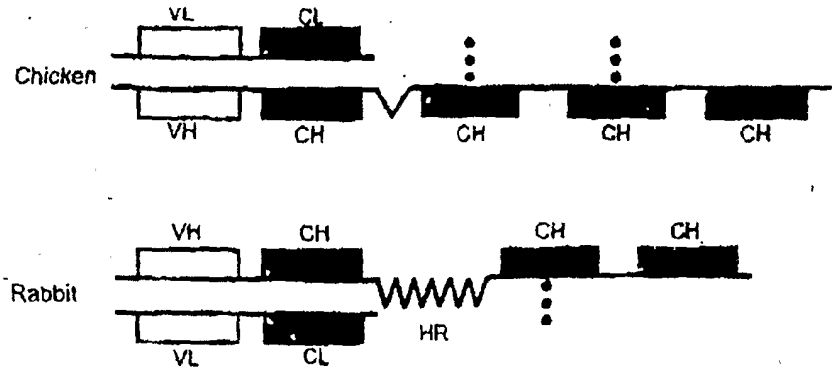

Figure 3

INTERNATIONAL SEARCH REPORT

PCT/IN 03/00434

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K16/02 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, PAJ, EMBASE, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 5 443 976 A (CARROLL SEAN B) 22 August 1995 (1995-08-22) column 13, line 57 - column 15, line 5; example 1	1,2 3-28
Y	US 5 871 939 A (GARSIA KIM ASTRID ET AL) 16 February 1999 (1999-02-16) column 3 - column 4	3
Y	WO 00/52055 A (KOBILKE HARTMUT) 8 September 2000 (2000-09-08) example 1	4,5
Y	US 5 118 610 A (KITTO G BARRIE ET AL) 2 June 1992 (1992-06-02) example 3	6-16, 26-28

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 November 2004	29/11/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Gruber, A

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2003/068786 A1 (KARANTH NITTURU GOPALAKRISHNA ET AL) 10 April 2003 (2003-04-10) page 3, paragraph 62 - paragraph 91; table 1	17-25
A	US 5 702 905 A (TAKAHASHI MIYOKO ET AL) 30 December 1997 (1997-12-30) example 3	1-28
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

ational application No.
PCT/IN 03/00434

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-6,15-28 (all partially), 7,8 (all completely)

   A process to produce and isolate insect-specific egg yolk antibodies, and use of these antibodies for ELISA, method to isolate insect protein, the insect being Acheta domesticus.

   ---

2. claims: 1-6,15-28 (all partially), 9,10 (all completely)

   A process to produce and isolate insect-specific egg yolk antibodies, and use of these antibodies for ELISA, method to isolate insect protein, the insect being Sitophilus oryzae.

   ---

3. claims: 1-6,15-28 (all partially), 11,12 (all completely)

   A process to produce and isolate insect-specific egg yolk antibodies, and use of these antibodies for ELISA, method to isolate insect protein, the insect being Rhyzopertha dominica.

   ---

4. claims: 1-6,15-28 (all partially), 13,14 (all completely)

   A process to produce and isolate insect-specific egg yolk antibodies, and use of these antibodies for ELISA, method to isolate insect protein, the insect being Tribolium castaneum.

INTERNATIONAL SEARCH REPORT

on patent family members

PCT/IN 03/00434

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