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**Original article**

## Mother moth examination for mulberry silkworms - to detect pebrine spores

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An important aspect of silkworm seed production is to ensure Pebrine disease free eggs. Pebrine is caused by the protozoa called *Nosema bombycis* and it is commonly observed in all the seasons but during the rainy and winter season, the infection level is more. It is transmitted to offspring by transovarial. After the female moths have laid their eggs they are subjected to microscopic examination to see if the moth is free from disease (pebrine) or not. Since pebrine spores are easily transmitted from diseased mother moths to the eggs, examination of the female mother moths must be carried out to ensure that their layings are free from disease.

### Mother moth examination:

- Adopt Fujiwara's method of sampling and mother moth examination to detect pebrine spores.
- Either green moths (egg laid fresh moths) or dry moths (dried at 70 °C for 6 hours) can be microscopically examined to detect pebrine spores at 600 x magnification.
- Green moths have to be examined immediately, while dry moths can be examined at a later date.
- Each smear has to be checked by two persons.

### Collection of samples for examination:

- Collect the mother moth samples on the next day of depairing 8:30 A.M.
- Follow the below mentioned sampling method for examination.

Day of emergence	Samples quantity for examination
First Day	All moths
Second Day	20%
Third Day	20%
Forth Day	All moths

**Procedure for mother moth examination :**

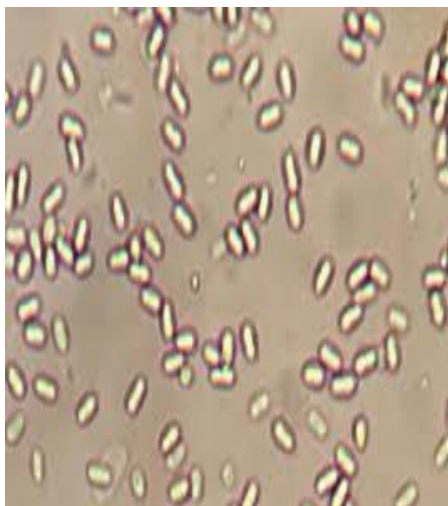
Steps	For green moth examination	For dry moth examination
1	Collect the samples as indicated in the above table.	Collect the samples as indicated in the above table.
2	Moths to be subjected for examination directly.	Dry the moths at 70 °C for 6 hours in oven.
3	Take 20 moths in a crushing jar and add 80 ml of 0.6 % K <sub>2</sub> CO <sub>3</sub> solution and crush for 2 minutes at 10000 rpm	Take 20 moths in a crushing jar and add 90 ml of 0.6 % K <sub>2</sub> CO <sub>3</sub> solution and crush for 2 minutes at 10000 rpm
4	Keep the jars for 2 minutes for the contents to settle.	Keep the jars for 2 minutes for the contents to settle.
5	Filter the contents into centrifuge tubes of 100 ml capacity through funnels with cotton and label them.	Filter the contents into centrifuge tubes of 100 ml capacity through funnels with cotton and label them.
6	Centrifuge the filtrate at 3000 rpm for 3 minutes.	Centrifuge the filtrate at 3000 rpm for 5 minutes.
7	Reject the supernatant solution.	Reject the supernatant solution.
8	Dissolve the sediment in 2~3 drops of K <sub>2</sub> CO <sub>3</sub> (0.6 %) solution and mix well using a cyclo-mixer for 10~15 seconds.	Dissolve the sediment in 2~3 drops of K <sub>2</sub> CO <sub>3</sub> (0.6 %) solution and mix well using a cyclo-mixer for 10~15 seconds.
9	Place the dissolved solution on a glass slide using glass rod to collect a thin smear.	Place the dissolved solution on a glass slide using glass rod to collect a thin smear.
10	Examine each smear over 5 microscopic fields to detect the presence of pebrine spores under 600 x microscopic magnification.	Examine each smear over 5 microscopic fields to detect the presence of pebrine spores under 600 x microscopic magnification.
11	Record scale of incidence if any as, 1~3 spores ± 4~10 spores 1 + 11~30 spores 2 + 31~100 spores 3 + 101~300 spores 4 + above 300 spores ∞	Record scale of incidence if any as, 1~3 spores ± 4~10 spores 1 + 11~30 spores 2 + 31~100 spores 3 + 101~300 spores 4 + above 300 spores ∞

**Characteristics of pebrine spore :**

- Pebrine spores appear as oval shining body, under 600x magnification of microscope.
- Pebrine spore looks round in polar view and has Brownian movement
- Phase contrast microscope testing of pebrine spore is most reliable.

**Prevention and Control of the Disease:**

- No control measure for the disease has yet been evolved. The elimination of diseased eggs during grainage by microscopic examination and diseased larvae during rearing is the only preventive measures against the disease.
- Follow the scientific inspection method of individual mother moth testing for detection of pebrine in egg production. Practice disinfection of grainage appliances before and after every grainage operation with 2% formalin.
- Ensure use of microscopically tested disease free disinfected eggs only.
- Practice surface sterilization of the eggs with 2% formalin for 5 minutes.
- Maintain hygienic conditions in egg production room and rearing sites.
- Practice disinfection of rearing appliances before use.
- During rearing, test the faecal matters, unequal/ lethargic/ unsettled/ irregular moulters periodically. If pebrine spores are detected, reject the entire infected crop.



PEBRINE SPORE



PEBRINE SPORE MICROSCOPIC OBSERVATION