# Avian Sample Submission Instructions



These instructions should be used in conjunction with the **Avian Sample Submission Form** which can be downloaded from our website.

## Choosing your sample type

Biobest is able to extract DNA from the widest possible range of sample types. Each test has specific sample options; please refer to the table below for further information and instructions.

Test	Egg Membranes	Mouth Swabs	Feather	Heparinised Blood	Faeces	Cloacal Swab	Tissue at PM
Sexing	$\sqrt{}$	$\sqrt{2}$	$\sqrt{}$	$\sqrt{}$			
Chlamydia				√1		$\sqrt{}$	
PBFD			$\checkmark$	$\sqrt{}$			V
Polyomavirus			$\checkmark$	$\sqrt{}$	1	$\sqrt{}$	V
Pigeon Circovirus				√3	$\sqrt{3}$	$\sqrt{3}$	<b>V</b>

<sup>&</sup>lt;sup>1</sup>There is no published data confirming that blood gives comparable results to faeces or tissue for Chlamydia.

**Egg Membrane:** Carefully scrape the membrane from the inside of the egg (or take a **small** section of the egg with the membrane attached) and place in a labelled container or sealable freezer bag. Please refrain from sending the whole egg or crushing the egg and membrane into tiny pieces. If storing samples for a few days before sending a batch, please allow the membrane to dry out before placing in container/bag, to prevent DNA degradation.

**Mouth Swab:** Ensure the bird has no food in its mouth; if necessary, rinse with water. Use only mouth swabs provided by Biobest – Please contact us. Remove swab from the tube (keep the lid attached) and rub the inside of the bird's cheek, on both sides, **firmly for at least 10 seconds**. Allow the swab to air dry for a few seconds. Place swab in the tube and push down the lid to close the tube. Pull the swab handle firmly away from the tube leaving the swab pad inside the tube. Close plug to seal tube. Label the tube clearly with the bird's reference.

It is possible to successfully extract DNA from egg membranes, feathers and blood in over 99% of samples. However it is harder to extract DNA from mouth swabs, not due to a test issue but due to sampling procedure, such that the successful extraction rate is about 60%. To maximise the chances of extraction success, please follow the sampling instructions very carefully and for mouth swabs read the additional information sheet.

**Feathers:** Pluck at least three feathers and place them in a labelled container or sealable freezer bag. Feathers should have follicle cells attached to them therefore mature chest feathers which come out easily are <u>not</u> the best to use. Newly emerging larger feathers are preferable as they contain feather pulp in their shafts. Clipped or moulted feathers cannot be tested. Please do not pluck primary, secondary or tail feathers.

**Blood:** It is advisable to use your veterinary surgeon for blood sampling in order to reduce the risk of contamination. If this is not possible blood can be collected by clipping the bird's toe nail however special tubes are required which contain heparin to prevent the blood clotting. About 50µl is required for all tests and one sample is enough to run any or all of the tests. If in doubt please consult your vet or consider an alternative sampling method.

Taking blood from birds which are sick or have poor clotting can be dangerous. If in doubt please consult your vet.

**Faeces:** Collect a small amount of fresh droppings and place in a labelled rigid leakproof container. A pooled sample, i.e. a small amount of 'rice-grain' droppings collected over 3-5 days, will provide optimum sensitivity. Larger volumes of one sample will not increase the chances of a positive result. It is not recommended to send samples from multiple birds as this will decrease the test sensitivity.

Cloacal Swabs & Tissue Samples: These sample types should only be taken by a vet.

<sup>&</sup>lt;sup>2</sup>On occasion it can be difficult to extract quality DNA from mouth swabs.

<sup>&</sup>lt;sup>3</sup>There is limited validation on these sample types.



# Submitting your sample

### **Submission Form:**

- Complete the submission form clearly and in full.
- Results are generally e-mailed.
- Make a record of the ID you have chosen for your bird. If you should need to contact us this will ensure we can quickly and easily locate your information.
- Please select the payment option, the preferred method is a payment link as this is the most secure option.
  With this option please ensure your contact details are legible. We can accept cash and cheques in pounds sterling if this is not suitable. If you are a regular user and wish to open an account please contact us
- Package your sample, submission form and payment (if cash/cheque chosen), a padded envelope is ideal.
- Ensure the correct postage has been applied and send the package to the address at the bottom of this form.

Please contact us if you require any advice or help with results. You can also visit our website (<u>www.biobest.co.uk</u>) which has further information and to download the appropriate forms.

# **Accuracy of DNA Sexing**

DNA testing is the method of choice for avian sex determination however it must always be remembered that no test system is perfect. There are several potential sources of error, each with slightly different implications.

**Sampling error:** Great care must be taken in collection and identification of each sample submitted to the laboratory. While we can take responsibility for testing a sample accurately, we cannot control sample collection. Where the laboratory repeatedly gives the same result for an original submitted sample this result is accurate whether or not it corresponds to the final sex of the bird judged by appearances or subsequent testing.

**Contamination:** In order to avoid cross-contamination, use fresh instruments and disposable gloves for each sample. If a sample is contaminated with material from other birds or mammals (including human) incorrect results may be obtained.

**Sample extraction:** It is possible to extract DNA from >99% blood, egg and feather samples, however only from 60% of mouth swab samples. Failure to extract DNA will mean that a new sample will have to be submitted and you will be charged for a second test.

**Laboratory error:** Biobest take great efforts to achieve 100% accuracy and have controls and checks in every test, however it is important that owners and breeders understand that no laboratory test achieves 100% accuracy. If you suspect a result may be inaccurate please contact us to discuss. In most instances we will be happy to provide a retest free of charge.

**Bird species:** Biobest utilise 3 slightly different DNA techniques to successfully sex the vast majority of bird species. Please contact us if you would like to check if we can sex a particular species as some species cannot be sexed by any of our methods.

**Individual variation:** The sexing technique relies on the precise DNA sequence of the individual bird. Even in a species that can normally be sexed we sometimes identify genetic lines where the sexing tests do not work or require slightly different conditions. Please contact us if you have any queries regarding this.

Many of the sources of error in sex determination can be minimised by careful attention to detail at all stages of the process from sampling to reporting of results. We aim to obtain the best possible level of accuracy but **would not** recommend that it is relied on as a sole measure to determine sex when birds are being sold. Where there is genuine concern that a result provided appears not to correspond with the apparent sex of the bird as it grows, we will happily discuss the case and test a repeat sample from the same bird free of charge to resolve the issue.