A Protocol for Basic Post-Mortem Examination and Sampling of the Cardiovascular System

Of Great Apes

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GUIDELINES

A Protocol for Basic Post-Mortem Examination and Sampling of the Cardiovascular System of Great Apes

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1. Introduction

This protocol has been created as part of the EAZA Great Ape TAG endorsed Ape Heart Project. Based at Twycross Zoo (UK), the project is a Europe wide collaborative initiative striving to achieve a better understanding of great ape cardiovascular disease through a combination of epidemiological study, clinical and pathological investigation.

This protocol aims to provide a guideline for performing a systematic and comprehensive approach to the post-mortem examination and sampling of the cardiovascular system in great apes.

Cardiovascular system examination should form part of a whole body gross examination and histopathology; this protocol is therefore intended to be supplementary to the general Great Ape TAG Veterinary Guidelines for performing post-mortem examination.

The purpose of this protocol is to;

- Promote consistency and quality in post-mortem examination of the cardiovascular system in great apes
- Standardise and maximise information gathering
- Facilitate comparative study between post-mortem findings and relevant samples
- Permit consistent sampling of the heart for subsequent examination by a designated pathologist

It is our ambition that all veterinarians and pathologists will follow these guidelines when performing post-mortem examination of great apes within EAZA collections.

If you still wish to use your own pathologist for examination of the heart, please refer them to us for a copy of our full cardiac post-mortem examination protocol.

When post-mortem examination of the cardiovascular system is performed, it is requested that photographs are taken at all stages of the process, and in particular of any abnormalities.

If the abnormal accumulation of fluid is noted at any stage, it should be quantified (in ml, or weighed if clotted), characterised (colour, consistency, specific gravity) and where possible a sample stored.
2. Identifying Information

All information requested in the sample submission form should be provided. All photos, paperwork and samples must be clearly labelled with patient identifying information, including the following:

- Studbook number
- Species (and subspecies if known)
- Individual institution ID (name/number)
- Zoological collection
- Date of birth
- Date of death

3. Supporting Documents/Files

A copy of the full post-mortem report or summary of the findings elsewhere in the body should also be sent, where possible. Other documents of use are:

- Copy of the animal’s clinical history
- A copy of the animal’s records (e.g. ARKS/ZIMS report)
- Photographs taken during the post-mortem examination;
  - Ideally photographs should be taken at all stages of the examination
  - Photographs of any abnormalities found are of particular use

4. Sending Samples

Once the heart is in formalin, please contact the project co-ordinator at the email address below. Once the heart is fixed, remove it from the formalin (this can be re-used) and wrap it in saline soaked gauze swabs or similar to prevent desiccation. Double bag the sample and place it into the container for postage/courier to the address below. Send all samples with a completed sample submission form.

If sending the sample from a zoological collection within the EU no CITES permit is required, but the sample should be accompanied by a letter detailing the nature of the contents (e.g. chimp post-mortem sample), and the reason for the transfer (i.e. research). If sending the sample from a zoo outside the EU additional advice from CITES should be sought.

5. Report of Findings

A preliminary report of macroscopic findings will be sent to the submitting zoo/vet within a week of receipt of the sample (by email or phone), and a full written report within 6 working weeks.
### Protocol for Post-Mortem Examination and Sampling of the Cardiovascular System of Great Apes

<table>
<thead>
<tr>
<th>STEP</th>
<th>ACTION</th>
<th>EXPLANATORY NOTE</th>
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| 1    | Weigh the animal  
Body condition score  
Measure crown-rump length | Record the weight (in kg)  
BCS scale 1-5  
From top of the head (crown) to the bottom of the buttocks |
| 2    | Open the chest and examine the thorax | Note appearance of lungs, pleural cavity etc.  
Assess for presence of lesions or fluid  
Take photos of the heart ‘in situ’ |
| 3    | Examine the pericardium | Assess for lesions, thickening or fluid (if present quantify, characterise and sample). Formalin fix the pericardium. |
| 4    | Remove the pluck  
Remove heart from pluck | Check the anatomy of the great vessels before sectioning (especially in young animals)  
Use a needle and syringe to draw blood from the right atrium before opening and freeze the sample as whole blood (-80°C if possible, -20°C otherwise)  
Cut the pulmonary trunk transversely 3cm above the pulmonary valve – assess the lumen for thrombi  
Transect all vessels as far from the heart as possible |
| 5    | Examine the epicardium | Note any thickening, lesions, changes in appearance, colour etc.  
Wash/rinse the heart before taking photos of the heart from all sides |
| 6    | Open the ventricles | Make a single transverse incision through the lower third of the apex perpendicular to the long axis of the heart to expose the chamber of both ventricles– see explanatory image overleaf  
Remove any clots and rinse the heart before weighing |
| 7    | Weigh the heart | Record the weight (in grams) |
| 8    | Sample the apical myocardium | Take one 1x1x0.5cm portion of the sectioned piece of apex and place in a universal tube for freezing (at -80°C if possible, or -20°C otherwise)  
If RNA later is available, also preserve an additional portion of myocardium approx. 3x3x3mm in size and immerse in fluid |
| 9    | Fix the heart | Fully submerge the heart in 10% neutrally buffered formalin ensuring all surfaces are covered and there is sufficient formalin around the heart  
Leave to fix for at least 48 hours. |
| 10   | Perform gross post-mortem examination of rest of carcass | Open the entire aorta along itself length to the level of the iliac bifurcation; sample and formalin fix any lesions  
Examine the remaining major body organs as per the GATAG post-mortem protocol and take relevant samples for histopathology.  
Take special note of the lungs, liver and kidneys and where possible also provide a formalin fixed sample (1x1x1cm) of these |
| 11   | Complete paperwork | Complete the sample submission form |
| 12   | Contact us | Email [heartproject@twycrosszoo.org](mailto:heartproject@twycrosszoo.org) |
| 13   | Send the heart | Refer to point 4 entitled “Sending Samples” above |

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**Ape Heart Project (EAZA Great Ape TAG Endorsed)**  
**Twycross Zoo, Burton Road, Atherstone, Warwickshire, UK, CV9 3PX**  
**Website: [www.twycrosszoo.org/ape-heart-project.aspx](http://www.twycrosszoo.org/ape-heart-project.aspx)**  
**Email: heartproject@twycrosszoo.org**

*Don’t forget to take photos at all stages of the cardiac post-mortem examination (include a scale marker)*
Figure 1:
Shows the approximate location of the transverse cut to be made across the lower third of the apex. The cut should be made perpendicular to the long axis of the heart approximately 4cm from the apex in gorillas and 3cm in the other three great ape species. A single cut at this location should expose the chambers of both ventricles, allowing clots to be removed prior to weighing and fixing.

Don’t forget to take photos at all stages of the cardiac post-mortem examination (include a scale marker)