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
- 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 fully fixed (depending on size 2-8 days), remove it from the formalin (this can be re-used) and wrap it in saline soaked gauzeswabs or similar to prevent desiccation. Double bag the sample and place it into a container for postage/courier to the address below. Send all samples with a copy of the completed sample submission form.

Each institution must obtain a CITES permit before samples can be sent, unless you are a CITES Registered Scientific Institution or you are able to send via one in your country (e.g., a university or museum you have a link with). For a full list of registered institutions please visit https://cites.org/eng/common/reg/e_si.html.

It may also be possible for sending institutions to apply for a CITES fee exemption (valid for several years) if obtaining a full permit, on the basis of the project’s contribution to research and conservation. Please contact your local CITES authority for more information.

Serum samples are very valuable to the research we are conducting on vitamin D levels and cardiac biomarkers. If possible, please send serum (0.5ml minimum) from the animal, even if this was taken months or years prior to death. Serum should be stored at -20°C until ready to send, and then it simply can travel with an ice pack or cooler.

5. Report of Findings

A full written report of macroscopic and histopathologic findings will be sent (by email) to the submitting zoo/vet within 8 working weeks of receipt of the sample. We are happy to update you on the progress or preliminary macroscopic findings in the meantime, just get in touch via email!
## 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 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 the pluck | Check the anatomy of the great vessels before sectioning (especially in young animals).  
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    | Take photos of the heart | 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. This should expose the chambers of both ventricles – see Figure 1 for clarification.  
This will ensure adequate formalin uptake into the myocardium.  
Remove any clots and rinse the heart before weighing |
| 7    | Weight the heart | Record the weight (in grams) |
| 8    | Sample the apical myocardium | If RNA Later is available, take a 3x3x3mm portion of the sectioned apical myocardium and immerse in fluid. This can be stored at +4°C and sent at room temperature (for several days at least before final storage in freezer) |
| 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 depending on size for 2-8 days |
| 10   | Perform gross post-mortem examination of rest of carcass | Open the entire aorta along its length to the level of the iliac bifurcation; sample and formalin fix any lesions.  
Examine the remaining major body organs as per the Great Ape TAG protocol and take relevant samples for histopathology.  
Take special note of the lungs, liver and kidney and where possible, also provide us with 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 samples | Refer to point 4 entitled “Sending Samples” above |

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 perpendicular to the long axis of the heart exposing the chambers of both ventricles. Please remove major post-mortem blood clots which may hinder adequate formalin fixation.

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

Ape Heart Project (EAZA Great Ape TAG Endorsed)
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Email: heartproject@twycrosszoo.org
Website: twycrosszoo.org/conservation/research-at-twycross-zoo/ape-heart-project/