HDBR
HUMAN DEVELOPMENTAL BIOLOGY RESOURCE

HDBR Fact Sheet

Fixation and Paraffin Wax Sections

Tissue is primary fixed in formalin phosphate buffered saline. After adequate fixation (time varies according to the thickness of the tissue) tissue is either processed, or secondary fixed in methacarn (60% methanol, 30% chloroform, 10 % acetic acid) at 4°C and stored until processing. Only embryos of the earlier stages (up to CS21) are fixed and processed whole. Later embryonic or fetal stages are too large to be fixed and processed whole and further dissection is required.

Tissue is then processed on a Shandon processor either on a short day program, or overnight, depending upon tissue size, through graded alcohols and several changes of xylene and paraffin wax. Processed tissue samples are then embedded in paraffin wax prior to sectioning.

Blocks of paraffin wax embedded tissue are sectioned on a microtome using Feather S35 disposable blades, usually at a thickness of between 7-9µ. Cut sections are then mounted on SuperFrost® Plus slides and dried overnight at 37°C, boxed and stored at 4°C.

Between 1 and 4 sections are mounted per slide, depending on the size of the tissue section. Every ninth section is taken and stained with haematoxylin and eosin in order to help with identification of anatomy and selection of stored unstained slides with the appropriate tissues for specific projects.

Digitised images of the haematoxylin and eosin stained sections are available to view online. Please contact hdbr@ncl.ac.uk for login details.

The slides we provide are mainly used for tissue *in situ* hybridisation (TISH) and immunohistochemistry and the sections will need to be de-waxed prior to use. Protocols for TISH and immunohistochemistry can be provided on request

Tissue can be sectioned in the sagittal, transverse or coronal planes.

HDBR contact details
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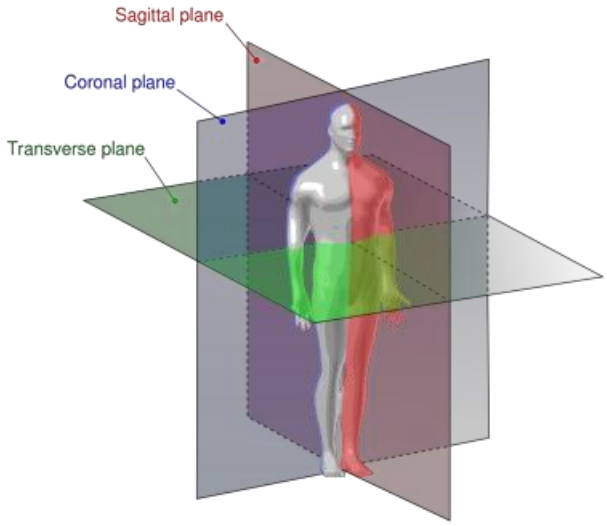
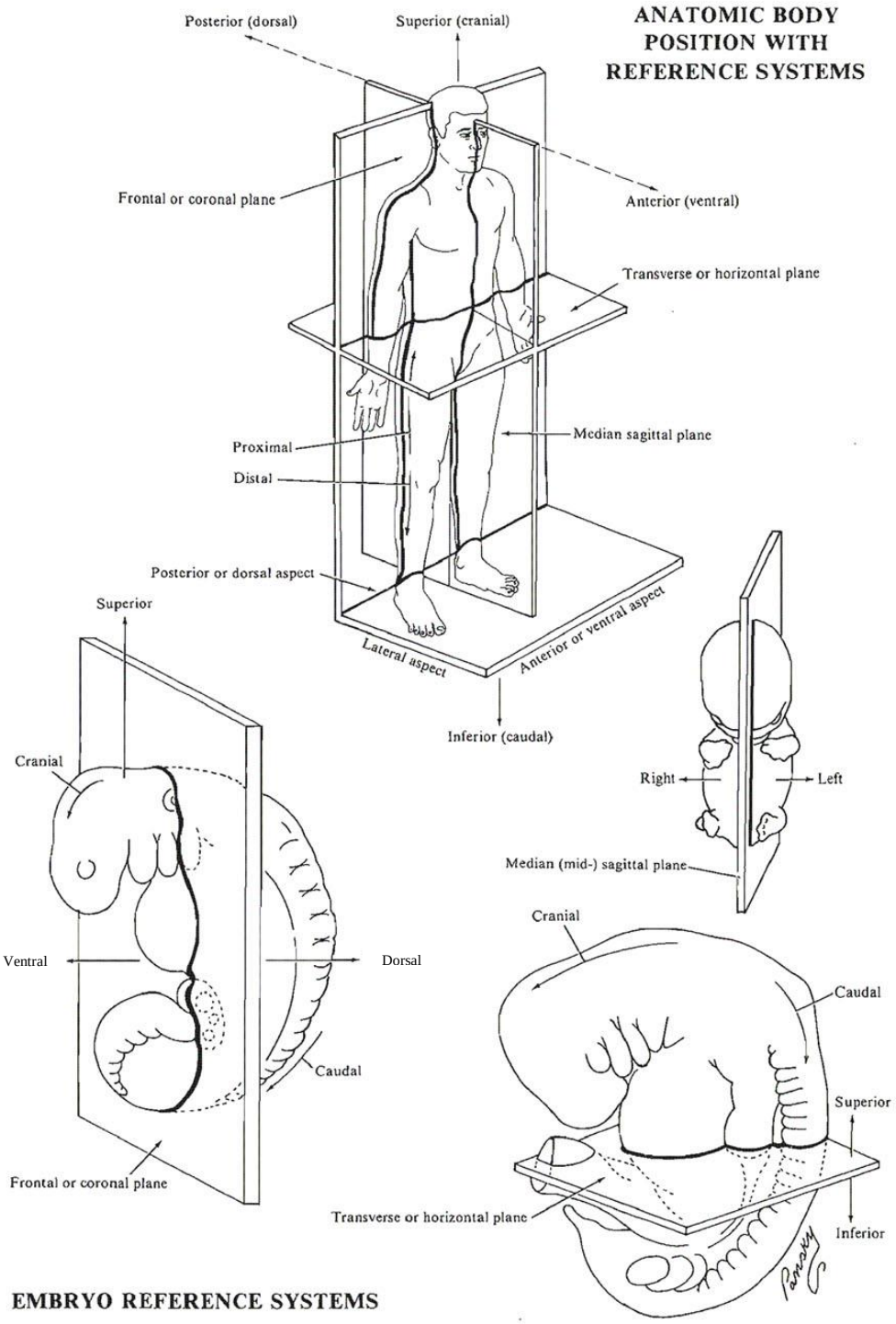
Paraffin Wax Embedded Embryo



https://www.boundless.com/physiology/textbooks/boundless-anatomy-and-physiology-textbook/introduction-to-human-anatomy-and-physiology-1/anatomical-terms-33/body-planes-and-sections-289-1344/



http://discovery.lifemapsc.com/

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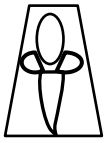
Sagittal

Transverse



Coronal

A slide from each cut block undergoes a quality control (QC) check, by immunohistochemistry or *in situ* hybridisation (TISH). Wax blocks can also be requested to be cut by the end user, but these are not QC checked. It is recommeded that slides are used within 6 months of receipt.

It is a requirement of HDBR project registration that all slides must be returned to HDBR at the end of the research project together with a completed submission form available at www.hdbr.org

Frozen Tissue Sectioning

Fresh as well as fixed tissue can also be sectioned. Tissue is embedded in OCT, either in a mould or on a piece of cork, either over dry ice or in a liquid nitrogen bath. Whole embryos are usually embedded left lateral and the mould marked accordingly, and can then be orientated in any plane to be cryosectioned (see above). Individual organs can be similarly embedded and sectioned.

Tissue is sectioned on a Leica CM3050 cryostat, using disposable Feather R35 blades.

Section thickness is determined by the registered user's requirements, for example immunohistochemistry, immunofluorescence or laser micro-dissection.

Slides with cryostat sections or frozen tissue blocks can be sent to registered users but these are not QC checked.

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