SOP Reference: BCNTB/SOP/002

Standard Operating Procedure for

Tissue processing for paraffin wax embedding

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Authorised by:

	Name	Designation	Signature	Date approved
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Document review history			
Version Number	Revision History	Name of Reviewer/Author	Review date
1.0	1st Issue	Uma Ekbote	January 2012
2.0	Admin edits, change to participating centres, sample processing changes	Rosie Robertson, Angie Berwick, Dr Fabricio Barros, Sameena Iqbal, Uma Ekbote	April 2016, December 2016, February 2017

1.0 PURPOSE AND SCOPE

1.1 Formalin fixed, paraffin embedding is a long-established method for preparing samples for histological procedures. These provide a sample with excellent morphological integrity and can be valuable source for studying features such as morphology and protein expression.

1.2 The process involves fixing the sample in the preservative, formalin, and then, through a series of graduated alcohol and subsequently xylene solutions (see appendices for details), impregnating the tissue with paraffin wax. This gives the tissue a structural integrity allowing thin sections to be cut on a microtome and the sections assessed using light microscopy. This tissue can also be further used for the construction of Tissue Micro Arrays (TMA).

2.0 DEFINITIONS

2.1 The Breast Cancer Now Tissue Bank shall be referred to as the Tissue Bank.

2.2 Material refers to any Tissue within the Tissue Bank.

2.3 Tissue refers to any tissue or fluid taken from the human body.

2.4 The Institutions are the University of Leeds, University of Nottingham, Barts Cancer Institute, Queen Mary University of London, University of Sheffield and University of Southampton.

2.5 Barts Cancer Institute Breast Tissue Bank is referred to as BCIBTB.

2.6 Tissue Microarray referred to as TMA.

3.0 REFERENCES

3.1 Control of Substance Hazardous to Health (COSHH) and Risk Assessments will be site-specific, adhering to national guidelines.

3.2 Human Tissue Authority, Codes of Practice and Standards, April 2017;

3.2.1 Code E: Research and Standards

3.3 BCNTB/SOP/001: Specimen Collection

4.0 HAZARDS AND PRECAUTIONS

All Members of staff must be signed up to the relevant University/NHS Control of Substances Hazardous to Health (COSHH) risk assessment protocol before carrying out this procedure.

4.1 **Vaccination:** Staff should have Hepatitis B vaccination under the guidance of Occupational Health Service.

4.2 **Fresh Tissue:** Unfixed tissue poses increased infection risk and gloves should be worn and standard laboratory practices followed.

4.3 **Formalin**: Moderately Toxic. If ingested may cause irritation to mouth, throat and stomach. Rinse mouth out thoroughly and drink plenty of water. Seek medical attention.

4.4 **Xylene:** Exposure to xylene can occur via inhalation, ingestion, eye or skin contact, and, to a small extent, by absorption through the skin. In the event of an accidental exposure, remove the victim from further exposure and seek medical assistance.

4.5 **Ethanol:** Avoid contact with skin and eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical assistance.

5.0 TISSUE PROCESSING AND EMBEDDING

5.1 With the exception of Leeds, the tissue processing and embedding SOP is managed by the site-specific NHS Trust histology laboratory. The processors at all sites are maintained by routine histopathology staff, therefore all SOPs pertaining to use and function will be the remit of routine histology.

5.2 The processing cycles vary depending on the equipment; however the end result is consistent in obtaining a good quality FFPE block. All stages involve high pressure and agitation to ensure thorough processing.

5.3 **Pre-processor steps for each site are as follows.**

5.3.1 **Leeds:** Samples are fixed in 10% neutral buffered formalin (Sigma HT501320-9.5l) for 24-48 hours to account for size of piece of tissue, at which point they are placed in 70% ethanol for up to 1 week before being processed. The processor at Leeds is run by the laboratory manager of the histology facility and samples are processed every Tuesday. **See Appendix A for Leeds details**.

5.3.2 **Nottingham:** Tissues are placed in 10% neutral buffered formalin for 12-24 hours (times noted) and processed overnight as detailed below. Samples are processed daily. **See Appendix B for Nottingham details**.

5.3.3 **Barts:** Whole specimens are sliced and placed in 10% neutral buffer for 24-48 hours (time recorded) at which point they are placed in 70% ethanol and processed routinely as overnight Program 1. See **Appendix C for Barts details.**

5.3.4 **Sheffield**: Samples are fixed in 10% neutral buffered formalin for 24-48 hours then processed overnight. **See Appendix D for Sheffield details.**

5.3.5 **Southampton:** Mastectomies tend to be sliced and left to further fix for 24 hours. Wide local excisions are sampled the day after receipt. Tumour cases for Bank are sampled following overnight fixation in

formalin. The cassettes are processed on an 18 hour overnight run.
See Appendix E for Southampton details.

6.0 APPENDIX A: LEEDS, LEICA APS 200

Station	Reagent	Time	Temperature
1	70% ethanol	30 min	37°C
2	80% ethanol	30 min	37°C
3	90% ethanol	30 min	37°C
4	95% ethanol	30 min	37°C
5	100% ethanol	1 hour	37°C
6	100% ethanol	1 hour	37°C
7	100% ethanol	1 hour 30 min	37°C
8	xylene	1 hour	37°C
9	xylene	1 hour 30 min	37°C
10	xylene	1 hour 30 min	37°C
11	wax	1 hour	65°C
12	wax	1 hour 30 min	65°C
13	wax	1 hour 30 min	65°C

Note: all steps undergo vacuum and agitation and 2 mins of draining.

7.0 APPENDIX B: NOTTINGHAM, LEICA ASP 300 PROCESSORS

OVERNIGHT FIXED (with formalin)						
Station	**Reagent**	**Time**	**Temp**	**Vac/Pres**	**Drain**	**Delay**
1	Formalin	00:51:00	A	Yes	140	Yes
2	70%IMS	01:00:00	A	Yes	140	No
3	IMS	01:00:00	A	Yes	140	No
4	IMS	01:00:00	A	Yes	140	No
5	IMS	01:00:00	A	Yes	140	No
6	IMS	01:00:00	A	Yes	140	No
7	IMS	01:15:00	A	Yes	140	No
8	Xylene	00:45:00	A	Yes	140	No
9	Xylene	01:00:00	A	Yes	140	No
10	Xylene	01:00:00	A	Yes	140	No
11	Wax	01:00:00	62	Yes	140	No
12	Wax	01:00:00	62	Yes	140	No
13	Wax	01:45:00	62	Yes	140	No

8.0 APPENDIX C: BARTS, LEICA ASP300 AUTOMATED VACUUM TISSUE PROCESSOR

Reagent	Duration (hours)	Temp (ºC)	P/V	Drain (sec)
Formalin	01.00	RT	P/V	120
Ethanol 70%	00.45	RT	P/V	120
Ethanol 90%	00.45	RT	P/V	120
Ethanol, Abs	00.45	RT	P/V	120
Ethanol, Abs	01.00	RT	P/V	120
Ethanol, Abs	01.00	RT	P/V	120
Ethanol/Xylene	00.30	RT	P/V	120
Xylene	00.45	RT	P/V	120
Xylene	01.00	RT	P/V	120
Xylene	01.30	RT	P/V	120
Histowax*	01.00	60	P/V	140
Histowax*	01.00	60	P/V	140
Histowax*	01.00	60	P/V	140

Key

P/V = Pressure Vacuum Cycle

Drain = Time taken to drain reagent from the retort.

Program duration = 12 hours 38 mins.

***:** Histowax is the commercial name for wax.

9.0 APPENDIX D: SHEFFIELD, Sakura VIP 5, Sakura VIP 6, Shandon Pathcenter, Leica Peloris, or Leica ASP300

Reagent	Time	Vacuum/Pressure	Temperature	Agitation
Formalin	10mins	On	Ambient	Fast
70% IMS	45mins	On	Ambient	Fast
95% IMS	45mins	On	Ambient	Fast
99% IMS	1hr 20mins	On	Ambient	Fast
99% IMS	1hr 20mins	On	Ambient	Fast
99% IMS	1hr 20mins	On	Ambient	Fast
99% IMS	1hr 20mins	On	Ambient	Fast
Xylene	1hr 30mins	On	Ambient	Fast
Xylene	1hr 30mins	On	Ambient	Fast
Xylene	1hr 30mins	On	Ambient	Fast
Paraffin Wax	1hr 30mins	On	60ºC	Fast
Paraffin Wax	1hr 30mins	On	60ºC	Fast
Paraffin Wax	1hr 30mins	On	60ºC	Fast
Paraffin Wax	1hr 30mins	On	60ºC	Fast

10.0 APPENDIX E: SOUTHAMPTON, Processing programme for breast tissue on Leitz Peloris processor

STATION	REAGENT	TIME	TEMP	P/V
1	Formalin	5	45	no
2	70% Alcohol	60	45	no
3	90% Alcohol	60	45	no
4	Absolute Alcohol	60	45	no
5	Absolute Alcohol	60	45	no
6	Absolute Alcohol	60	45	no
7	Absolute Alcohol	60	45	no
8	Absolute Alcohol	120	45	no
9	Xylene	60	45	no
10	Xylene	60	45	no
11	Xylene	120	45	no
12	Wax	90	65	yes
13	Wax	120	65	yes
14	Wax	180	65	yes