

Tumour BRCA (tBRCA) guidelines for pathologists are extrapolated from recommendations for HER-2 testing. There are no published studies on pathology protocols and result outcomes in tBRCA testing. This guidance is based on general principles and author experience.

Tubo-ovarian cancer and BRCA

The frequency of *BRCA1* and *BRCA2* germ-line pathogenic variations (mutations) in women with tubo-ovarian cancer is variably quoted. If all tubo-ovarian cancers are taken into consideration, the stated frequency is 10 – 15%. [Alsop K, Fereday S, Meldrum C, deFazio A, Emmanuel C, George J, Dobrovic A, Birrer MJ, Webb PM, Stewart C, Friedlander M, Fox S, Bowtell D, Mitchell G. *BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women with ovarian cancer: a report from the Australian Ovarian Cancer Study Group. J Clin Oncol. 2012 Jul 20;30(21):2654-63. doi: 10.1200/JCO.2011.39.8545.*] When somatic mutations are included, this figure rises to 20% or greater. [Ledermann JA, Drew Y, Kristeleit RS. *Homologous recombination deficiency and ovarian cancer. Eur J Cancer. 2016;60:49-58. doi:10.1016/j.ejca.2016.03.005.*]

Recombinant DNA Repair

Recombination occurs when two molecules of DNA exchange pieces of genetic material with each other. This must be accurate in order to maintain genetic integrity. The most notable example of recombination is in meiosis resulting in creation of gametes that contain new combinations of parental genes. Throughout life, the DNA undergoes damage. There are six major DNA repair pathways in humans. These include base excision repair, nucleotide excision repair, single strand break repair, homologous recombination (HR) repair, non-homologous end joining and mismatch repair. [Jackson SP, Bartek J. *The DNA-damage response in human biology and disease. Nature. 2009;461(7267):1071-1078. doi:10.1038/nature 08467.*] The HR pathway consist of a set of related sub-pathways that utilize DNA strand invasion and template-directed DNA repair synthesis to effect a high-fidelity repair of damaged DNA.

Recombinant DNA repair and BRCA pathogenic variants

The HR pathway involves the coordinated interactions of many proteins including *BRCA1* and *BRCA2* and other proteins such as *RAD51* and proteins of the Fanconi anaemia pathway. Alterations of the *BRCA1* and *BRCA2* genes may occur as a germline abnormality, but may also occur through mechanisms such as somatic mutations and epigenetic silencing. [Moschetta M, George A, Kaye SB, Banerjee S. *BRCA somatic mutations and epigenetic BRCA modifications in serous ovarian cancer. Ann Oncol. 2016;27(8):1449-1455.*] Deficiency in HR is a target for Poly(ADP-ribose) polymerase (PARP) inhibitors.

Germline mutations vs somatic mutations

Germline mutations are inherited mutations and are present in every cell of the body. Somatic mutations are non-inheritable mutations that are found only in tumour cells. Upto 6-7% of high grade serous tubo-ovarian carcinomas have somatic *BRCA1/2* mutations. tBRCA and somatic *BRCA* are not synonymous. *BRCA* mutations in tumour cells reflects both germline and somatic mutations.

Testing for BRCA pathogenic variants (mutations)

Germline testing is generally done using blood. tBRCA testing is done mostly by using formalin fixed paraffin embedded (FFPE) tissue from the carcinoma. Cytology samples, rarely, can also be used.

Reasons for tBRCA testing

PARP inhibitors inhibit DNA repair pathways and cause apoptosis/death of cancer cells, especially in HR-deficient cells. tBRCA testing is important to identify this subgroup of patients. Tumours harbouring BRCA1/2 mutations (detected by tBRCA testing) in the tumour, irrespective of germline or somatic, are also associated with better response to platinum-based chemotherapy. tBRCA abnormalities due to germline BRCA mutations have additional implications in identifying BRCA germline mutation carriers.

Role of the pathologist

The pathologist plays an important role in selection of the test sample and is the member of the multidisciplinary team who has access to pre-test and post-test pathways and is pivotal in establishing standard operating procedures, audit of the process and institution of change if needed.

Understanding pre-analytic variables

The process of acquiring tissue starts with tissue collection by the clinician as a diagnostic or resection sample. Warm ischemia time is the time from the interruption of the blood supply to the tumour to the excision of the tissue specimen. This is followed the cold ischaemia time which is the time taken to transfer the surgical specimen into the fixative. The length of this time influences the levels of gene expression and is an important factor. The cold ischaemia time is less for small samples acquired at inpatient or outpatient settings.

Once in the specimen container, the tissue is penetrated by the fixative before the actual process of fixation starts. This is a problem particularly with large specimens such as ovarian tumours. [Goldstein NS, Hewitt SM, Taylor CR, Yaziji H, Hicks DG. *Recommendations for Improved Standardization of Immunohistochemistry.* *Applied Immunohistochemistry & Molecular Morphology* 15 (2007): 124-133].

The fixative of choice is 10% neutral buffered formalin. Formalin should only be used for upto 24 h after dilution to 4% w/v. After 24 hours, polymerisation starts and a stable pH and 4% concentration gets affected. Formalin penetrates tissue at around 1 mm/hour. A minimum of 6 hours of formalin fixation is required, complete tissue fixation requires up to 24 hours. Prolonged fixation (arbitrarily designated as beyond 36 hours) is a possible cause for test failure and should be averted wherever possible. Fixation over the weekend, especially of small biopsies, should be avoided.

Laboratory processes

Sections should be cut under conditions (clean microtome etc) that avoid cross contamination from other specimens.

Appropriate numbers of air dried, mounted, unstained, non coverslipped sections should be sent.

For cytology specimens, It is essential that cells and tissue fragments from the cytology samples are processed into agar/cell blocks, formalin-fixed and paraffin embedded and then undergo an assessment process as per tissue samples.

The request forms

Requests for tBRCA testing can be made by managing clinicians, nurse specialists, multidisciplinary teams or pathologists. This is a local decision. In all scenarios, patient consent needs to be confirmed and documented.

At the time of writing these guidelines, the BRCA form testing form (Astra Zeneca) can be downloaded from

<https://medicines.astrazeneca.co.uk/content/dam/multibrand/uk/en/resources/lynparza/tbrca-testing/0715-test-request-form-Manchester.pdf>

<https://medicines.astrazeneca.co.uk/content/dam/multibrand/uk/en/resources/lynparza/tbrca-testing/0715-test-request-form-Royal-Marsden.pdf>

The results should be requested to generic pathology and generic clinical emails.

Choosing material for testing

In England, tBRCA testing is advised for high grade serous carcinomas and in Scotland tBRCA testing is advised for high grade serous and endometrioid carcinomas. The diagnosis is made in several settings. The pathologist or advanced practitioner dealing with the specimen may not be a specialist in gynaecological pathology. This guideline advises the following in order to conserve the maximum amount of tissue for tBRCA test.

Biopsy of suspected tuboovarian carcinoma:

- Cores blocked separately (at least 2 blocks)
- H&E on both blocks to confirm cancer
- One block (preferably the one with less tissue/tumour) for confirmatory IHC
- IHC to confirm high grade serous carcinoma (PAX8+ve, WT1 +ve, ER +ve. p53 mutation/aberrant) (<https://www.thebagp.org/download/bagp-uknegas-project-p53-interpretation-guide-2016/>) If there is diagnostic uncertainty, in order to preserve tissue for testing, further immunostains should not be done. The available material should be sent to Cancer Centre for review and diagnosis.
- Tissue/blocks, H&E and immunostained slides should be sent to nominated pathologist.

Resection specimen from known high grade tuboovarian serous carcinoma:

- Reporting pathologist should send block/tissue from primary or metastatic carcinoma containing maximum viable and well-fixed tumour and its H&E stained slide to nominated pathologist

Cell block from fluid sample (pleural effusion or ascites) in suspected tuboovarian carcinoma:

- H&E to confirm cancer
- Minimal IHC to confirm high grade serous carcinoma (PAX8+ve, WT1 +ve, ER +ve. p53 mutation/aberrant)
- Block, H&E and IHC slides sent to nominated pathologist.

Sending material for testing

Nominated pathologist/s mark tumour areas on H&E slide and estimates tumour volume within the whole section and within the marked areas. As a guidance, the marked areas should contain at least 20% tumour cells.

The tissue block, the marked slide and the completed form are sent to the appropriate genetic laboratory hub.

Recording the report

When the result is received, it should be added (in full) to the initial pathology report as a supplementary. Wherever possible, the pathologist should ensure including the result in the MDT and patient records and make the result accessible to the managing clinician. Local pathways should be followed

Audit

We recommend that there should be mechanisms in place to document preanalytic variables, laboratory processes and tumour content prospectively to enable audit of these parameters.