Appendix 2 – Pathological examination of the testis

Mandatory pathological requirements:

- *Macroscopic features:* It must indicate radical or partial orchidectomy, side, testis size, number of tumours, and macroscopic features of the epididymis, cord length, and tunica vaginalis.
- *Sampling:* At least a 1 cm^2 section for every centimetre of maximum tumour diameter including normal macroscopic parenchyma (if present), tunica albuginea and epididymis, with selection of suspicious areas. If the tumour is < 20 mm it should be completely sampled.
- At least one proximal (base of the cord) and one distal section of spermatic cord plus any suspicious area. Cord blocks should preferably be taken prior to tumour sections to avoid contamination.
- *Microscopic features and diagnosis:* Histological types (specify individual components and estimate amount as percentage) according to WHO 2022 [1]:
  - -- Presence or absence of peri-tumoral lymph and/or blood vessel invasion. In case of doubt, the use of endothelial markers, such as CD31, are recommended.
  - -- Presence or absence of GCNIS in non-tumour parenchyma.
  - -- In case of rete testis invasion attention should be paid to distinguishing between pagetoid involvement and stromal invasion [2].
- If microscopic findings are not concordant with serum markers further block samples should be taken.
- Pathological tumour (pT) category according to TNM 2016 [3]. In a multifocal seminoma the largest nodule should be used to determinate pT category.

Immune-histochemical markers in cases of doubt are:

- Seminoma: CD-117 (c-KIT), OCT 3/4, Sall4, PLAP
- GCNIS: CD-117 (c-KIT), OCT 3 / 4, Sall4, PLAP
- Syncytiotrophoblastic: β-hCG
- Embryonal carcinoma: CD30
- Yolk sac tumour: Glypican 3, AFP.
- Sex cord gonadal tumours: Inhibin, calretinin steroidogenic factor 1.

The search for i12p (FISH or PCR) or gain in Chr9 (spermatocytic tumour) are additional molecular techniques which are only rarely required. Confirmation of the utility of other molecular markers such as P53, MDM2, KRAS and HRAS is awaited [4].

In order to facilitate consistent and accurate data collection, promote research, and improve patient care, the International Collaboration on Cancer Reporting has constructed a dataset for the reporting of urological neoplasms. The dataset for testicular tumours encompasses the updated 2016 WHO classification of urological tumours, the ISUP consultation and staging with the 8th edition of the American Joint Cancer Committee (AJCC) [5].

The dataset includes those elements unanimously agreed by the expert panel as “required” (mandatory) and those “recommended” (non-mandatory) that would ideally be included but are either non-validated or not regularly used in patient management [5]. The dataset for handling pathological assessment of TC is shown in Table 1.

Table 1: Recommended dataset for reporting of neoplasia of the testis (modified from the International Collaboration on Cancer Reporting) [5].

Elements	Required	Recommended*	Content	Remarks
Clinical information		√	- Not provided - Previous history of testicular cancer - Previous therapy - Other	Specify each
Serum tumour markers		√	- Not provided - If provided within normal limits or - Specify serum tumour markers used - Specify levels - Specify date markers were drawn	Select all that apply: Serum tumour markers: LDH (IU/L), AFP (ug/L), β-hCG (IU/L)
Operative procedure	√		- Not specified - Orchidectomy partial - Orchidectomy radical - Other	Specify side for partial or radical orchidectomy. Specify other
Tumour focality	√		- Cannot be assessed - Indeterminate - Unifocal - Multifocal	If multifocal specify number of tumours in specimen.
Maximum tumour dimension	√		- Cannot be assessed - Dimensions largest tumour (mm)	Specify at least maximum diameter of largest tumour.

			- Dimensions additional tumour nodules[#]	Preferably specified 3 dimensions/axes[#].
Macroscopic extent of invasion	√		- Cannot be assessed - Confined to testis - Invades epididymis - Invades tunica vaginalis - Invades hilar structures - Invades spermatic cord - Invades scrotum - Other	Select all that apply. If other specify.
Block identification key		√	N/A	List overleaf or separately with indication of nature and origin of all tissue blocks.
Histological tumour type	√		- Germ cell tumour: type and percentage - Other	Use WHO classification (2022) update [1]. If other specify.
Microscopic extent of invasion	√		- Rete testis of stromal/ interstitial type - Epididymis - Hilar fat - Tunica albuginea[#] - Tunica vaginalis	For all: - not submitted - not involved - involved

			- Spermatic cord - Scrotal wall	
Lymphovascular extension	√		- Not identified - Present	If present specify type[#].
Intratubular lesions (GCNIS)	√		- Not identified - Present - Other intratubular lesions[#]	If other intratubular lesions present identify type[#].
Margin status	√		- Partial orchidectomy: . cannot be assessed . involved . not involved - Radical orchidectomy: . cannot be assessed . spermatic cord margin involved . spermatic cord margin not involved - Other margin involved	In partial orchidectomy if margin not involved, distance of tumour from closest margin (mm)[#]. If other margin involved specify.
Coexisting pathology		√	- None identified - Hemosiderin-laden macrophages - Atrophy - Other	If other specify.
Ancillary studies		√	- Not performed - Performed	If performed specify.

Response to neoadjuvant therapy		√	- Present - Absent - No prior treatment - Cannot be assessed	Explain reasons if cannot be assessed.
Pathologic staging*	√		T classification according to TNM 8th edition (UICC)**	m-multiple primary tumours r-recurrent y-post-therapy

* *Not mandatory. Ideally to be included but either non-validated or no regularly used in patient management.*

** *TNM 8th edition (AJCC) used in the original publication.*

\# *Recommended, i.p. intratubular seminoma and embryonal carcinoma.*

References:

1. Moch, H., et al. The 2022 World Health Organization Classification of Tumours of the Urinary System and Male Genital Organs-Part A: Renal, Penile, and Testicular Tumours. Eur Urol, 2022. 82: 458. https://pubmed.ncbi.nlm.nih.gov/35853783/

2. Verrill, C., et al. Reporting and Staging of Testicular Germ Cell Tumors: The International Society of Urological Pathology (ISUP) Testicular Cancer Consultation Conference Recommendations. Am J Surg Pathol, 2017. 41: e22. https://pubmed.ncbi.nlm.nih.gov/28368923/

3. Brierley, J.E., et al., The TNM Classification of Malignant Tumours 8th edition. 2016.

4. Kuczyk, M.A., et al. Alterations of the p53 tumor suppressor gene in carcinoma in situ of the testis. Cancer, 1996. 78: 1958. https://pubmed.ncbi.nlm.nih.gov/8909317/

5. Berney, D.M., et al. Datasets for the reporting of neoplasia of the testis: recommendations from the International Collaboration on Cancer Reporting. Histopathology, 2019. 74: 171. https://pubmed.ncbi.nlm.nih.gov/30565308