

Appendix 9. Review of teratoma methodologies used in different laboratories

18 laboratories from 16 countries responded to a set of questions (see table) on the teratoma assays they used for evaluation of hESC lines. Methodologies used for teratoma assays showed little concordance for strain of mouse, site of injection, number of cells/volume, end point, inspection for metastasis and processing and analysis of tumors (data obtained by S Oh and L Healy of ISCB).

Method component	Details requested	Range of methods reported
Preparation of hPSCs	Culture method used prior to harvesting	Feeder and feeder-free cultures used (some labs used both methods).
	Harvest method used	4 methods used (TrypLE, trypsin, collagenase, mechanical)
	Post-harvest processing before inoculation	3 methods used (cell centrifuged and resuspended, cells washed in growth medium or PBS and resuspended in the same, cells resuspended in medium with Matrigel™)
Cells inoculated	Number and viability of cells inoculated	Range of methods based on cell number (1000 -10,000,000), cell viability (range 80–95%), injection volume 20–150ul, and no determination of cell number or viability.
Site of inoculation	Anatomical site and means of administration (e.g. syringe, surgical incision with cells on a substrate)?	4 different sites used (legs intramuscular (both sides), Kidney capsule, intra-testicular, subcutaneously head and neck and flank)
Test animal	Strain of mouse used	Seven different strains of mouse used (Nude Balb/c, SCID undefined, Nude/nude, SCID/Beige, SCID c gamma c -/-, SCID undefined, NOD/SCID, NOD/MrkBomTac-Prkdc SCID).
	Frequency and natural onset of spontaneous tumors in the mouse strain	None identified or 'low'
	Age of animals used	2 age ranges used (7–8 weeks (majority) or 5–8 months)
Replicates of test	No. of animals used for each test	Ranged from 1–4 per cell line (3/cell line (majority), 1/cell line with 2–4 injection sites, 4/cell line)
	Observation of animals	Typical number of weeks post inoculation at which mice are sacrificed
Tumor incidence	Is there a maximum end point for incubation or are mice kept until natural death?	4–12 weeks
	Frequency of mice developing tumors per experiment	3 different limits used (2–4 months, tumor growth allowed to reach 1–1.5 cm or tumor growth allowed to reach 2 g)
	Method of tumor location	Ranged from none-100% with an equal number of participants reporting incidence of tumors in mice at 30–50% and 80–100%.
Tumor preparation	Numbers of tumors expected per mouse	Palpation and observation
	Frequency of metastasis	1–2 tumors per site
	Point at which palpable tumors are recovered	Majority of participants reported metastases
	Processing of tumor	5–12 weeks or maximum size of 0.5–1.5 cm or maximum weight of 2 g
Evaluation and reporting of tumors	Minimum criteria (in terms of histological data) to establish a cell line is 'pluripotent'	Tumors fixed by alternative methods (paraformaldehyde or formalin, paraffin, cryosections) depending on post-fixation testing including 3 different techniques (histology [H&E, PAS etc], immune-staining or PCR).
	Are results from more than one mouse used in combination?	Evidence of 3 germ layers
	Variation in results observed between cell lines	50% responded 'no', 50% responded 'yes' if using the same cell line
		Of those responding 50% reported no variation and 50% did see a significant variation

