Focus on: IMPLANT TESTING
ISO 10993-6

Implantation

- Assess the local pathological effects on living tissue, at both the gross level and microscopic level,
  - Sample of a material or final product that is surgically implanted or placed in an implant site or tissue
  - Appropriate for the site, route and duration of contact.

Scope: materials 1/2

- Solid and non-biodegradable;
  - Dental implants
  - Cardiac valves
  - Pacemakers
- Non-solid, such as porous materials, liquids, pastes and particulates.
  - Scaffolds for bone growth
  - Wound dressing
  - Fillers in putty (injectable)

Scope: materials 2/2

- Degradable and/or resorbable;
  - Resorbable bone scaffolds
  - Resorbable stitches sutures
  - Fillers
- Evaluate particulates, degradation products

Aim

- Characterize the history and evolution of the tissue response after implantation
- As compared to a known (accepted, state of the art) positive control and if possible a negative control (void)
- NOT intended to evaluate or determine the performance of the test specimen
  - Mechanical performance
  - Functional loading

Planning of tests

- Animal model:
  - species: usually rats or rabbits, larger animals must be justified
  - site of implant as appropriate to the kind of device: bone, tissue, subcutaneous
  - number of specimens per animal: lower number of animals, avoid cross-effects
- Control
  - Positive: state of the art, market competitor, predicate device
  - Negative: void, inert material, …
- Size of implant specimen
  - Proportionate to animal size? Full device? Miniaturized device?
- Pre-implant procedures i.e. mixing, polymerization, insert in holders, seeding with cells as appropriate (avoid immune reactions?)

Test period

- Required time points:
  - no or minimal degradation, usually to be evaluated at 1 week to 12 weeks after implantation;
  - while degradation is taking place;
  - when a steady state has been reached (tissue restoration or degradation nearing completion)
- Animals should be killed at each time point, in line with ISO 10993-2. Serial harvest under general anaesthesia with recovery may be acceptable under special circumstances, which shall be documented and justified.

Test period choice

Table 1 — Selection of test periods for long-term implantation

Species	Implantation period in weeks				
	12	26	52	78	(104) [a]
Rats	X	X	X		
Guinea pigs	X	X	X		
Rabbits	X	X	X	X	X
Dogs	X	X	X	X	X
Sheep	X	X	X	X	X
Goats	X	X	X	X	X
Pigs	X	X	X	X	X

[a] Depending on the intended use of the test material, not all implantation periods may be necessary (see ISO 10993-12). An observation period of 104 weeks may be of interest in selected instances.

Surgery and testing- subcutaneous

- Specimens: flat and thin, membranes or tubes (10 mm in diameter or lenght)
- Subcutaneous insertion must avoid doubling or wrinkling of sheet
- Preferred the dorso or the neck
- At least three animals, a total of 10 test and 10 control samples for each material and implantation period. Sections for histology shall be at least 1 cm apart.

Surgery and testing- muscle

- Specimens: pod-shaped, cilinders, no rough ends or sharp parts (10 mm long)
- Insertion completely in the muscle
- Paravertebral muscles of rabbits or gluteal muscle of rats
- At least three animals, a total of 10 test and 10 control samples for each material and implantation period.

Surgery and testing -bone

- Specimens: no predefined shape, preferred cylinder; size from 2 to 12 mm depending on species
- Complete or partial insertion according to intended use
- Cancellous (“spongy”) or dense compact bone of rabbits, dogs, sheep, goat, pig

Macroscopic Results

- Macroscopic assessment
  - Of implant site
  - Of lymphnodes
  - Of animal carcass if appropriate
  - Gross evaluation of haematoma, oedema, encapsulation and/or additional gross findings
  - MUST take pictures
- No predefined pass-no pass index is given in the norm
  - Comparison to the controls to assess risk

Microscopic Results: biological response

- Tissue
  - fibrosis/fibrous capsule (layer in micrometres) and inflammation;
  - changes in tissue morphology;
  - presence, extent and type of necrosis;
  - other tissue alterations such as vascularization, fatty infiltration, granuloma formation and bone formation;
- Cells:
  - number and distribution as a function of distance from the material/tissue interface of the inflammatory cell types, namely polymorph nuclear neutrophilic leucocytes, lymphocytes, plasma cells, eosinophils, macrophages and multinucleated cells;
- NOTE: Adverse histological responses shall be documented by photomicrograph.

Microscopic Results: material

- fragmentation and/or debris presence
- form and location of remnants of degraded material;
- quality and quantity of tissue ingrowth, for porous and degradable implant materials.
  - % of new tissue
  - % of remaining implant material

Microscopic Results: material

- For degradable/resorbable materials, at the intermediate or nearly complete degradation levels,
  - Evaluate quantity and state of the residuals
  - Evaluate of the restoration to normal structure
- For implants in bone,
  - Evaluate the area of bone contact and the amount of bone in the vicinity of the implant
  - Evaluate new non-calcified tissue, bone resorption or new bone formation

Results scores: cells

Table E.1 — Examples of a histological evaluation system — Cell type/response

Cell type/response	Score				
	0	1	2	3	4
Polymorphonuclear cells	0	Rare,1-5/phf [a]	5-10/phf	Heavy infiltrate	Packed
Lymphocytes	0	Rare,1-5/phf	5-10/phf	Heavy infiltrate	Packed
Plasma cells	0	Rare,1-5/phf	5-10/phf	Heavy infiltrate	Packed
Macrophages	0	Rare,1-5/phf	5-10/phf	Heavy infiltrate	Packed
Giant cells	0	Rare,1-2/phf	3-5/phf	Heavy infiltrate	Sheets
Necrosis	0	Minimal	Mild	Moderate	Severe

[a] phf = per high powered (400 ×) field.

Results scores: tissue

Table E.2 — Examples of a histological evaluation system — Response

Response	Score				
	0	1	2	3	4
Neovascularisation	0	Minimal capillary proliferation, focal, 1-3 buds	Groups of 4-7 capillaries with supporting fibroblastic structures	Broad band of capillaries with supporting structures	Extensive band of capillaries with supporting fibroblastic structures
Fibrosis	0	Narrow band	Moderately thick band	Thick band	Extensive band
Fatty infiltrate	0	Minimal amount of fat associated with fibrosis	Several layers of fat and fibrosis	Elongated and broad accumulation of fat cells about the implant site	Extensive fat completely surrounding the implant

Results acceptance

Conclusion: Under the conditions of this study, the test sample was considered a

- non-irritant (0,0 up to 2,9)
- slight irritant (3,0 up to 8,9)
- moderate irritant (9,0 up to 15,0)
- severe irritant (> 15)

to the tissue as compared to the negative control sample.

Use of implant testing for..

- Performance assessment
  - Time of degradation or integration
  - Trauma on local tissues
  - Integration scores (detachment)
- Preclinical assessment
  - Clinical parameters
- Predicate device comparison
  - Used as control

Performance assessment

- Expected technical features of implant
  - Change of physical characteristics over time
  - Stress test
  - Surface characterization
- Expected in vivo behaviour
  - Degradation, particles
  - Cracks, crevices

Preclinical assessment

- Clinical parameters
  - Osteointegration or integration in tissue
  - Presence of fibrous or healthy tissue
  - Different behavior at the interface of different tissues (example: dental implant with bone and gum)
- Time of healing, pain and swelling, infection

Predicate device as (additional) control

- Defines “state of the art” behavior
- Equivalent clinical outcome in vivo helps confirm clinical equivalence
  - Literature on predicate acceptable as appropriate
  - Lower need of clinical trials
- Better clinical trial planning
  - Exclude potential clinical risks
  - Better define clinical trial endpoints

Questions?