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

<table>
<thead>
<tr>
<th>Species</th>
<th>Implantation period in weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Rats</td>
<td>X</td>
</tr>
<tr>
<td>Guinea pigs</td>
<td>X</td>
</tr>
<tr>
<td>Rabbits</td>
<td>X</td>
</tr>
<tr>
<td>Dogs</td>
<td>X</td>
</tr>
<tr>
<td>Sheep</td>
<td>X</td>
</tr>
<tr>
<td>Goats</td>
<td>X</td>
</tr>
<tr>
<td>Pigs</td>
<td>X</td>
</tr>
</tbody>
</table>

* 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 length)
- 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, cylinders, 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>
<thead>
<tr>
<th>Cell type/response</th>
<th>0</th>
<th>1</th>
<th>Score</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymorphonuclear cells</td>
<td>0</td>
<td>Rare,1-5/phf a</td>
<td>5-10/phf</td>
<td>Heavy infiltrate</td>
<td>Packed</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0</td>
<td>Rare,1-5/phf</td>
<td>5-10/phf</td>
<td>Heavy infiltrate</td>
<td>Packed</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>0</td>
<td>Rare,1-5/phf</td>
<td>5-10/phf</td>
<td>Heavy infiltrate</td>
<td>Packed</td>
</tr>
<tr>
<td>Macrophages</td>
<td>0</td>
<td>Rare,1-5/phf</td>
<td>5-10/phf</td>
<td>Heavy infiltrate</td>
<td>Packed</td>
</tr>
<tr>
<td>Giant cells</td>
<td>0</td>
<td>Rare,1-2/phf</td>
<td>3-5/phf</td>
<td>Heavy infiltrate</td>
<td>Sheets</td>
</tr>
<tr>
<td>Necrosis</td>
<td>0</td>
<td>Minimal</td>
<td>Mild</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
</tbody>
</table>

a phf = per high powered (400 ×) field.
### Table E.2 — Examples of a histological evaluation system — Response

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