Pre-clinical In Vivo Testing Methods For Medical Devices

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Presentation Outline

» Review of testing based on type of contact and contact duration
» Extraction procedures
» Current in vivo test methods
ISO 10993-1: Device Categories

» Type of body contact
  ▪ Surface contacting devices
  ▪ Externally communicating devices
  ▪ Implant devices
ISO 10993-1: Device Categories

Contact Duration

- Limited: \( \leq 24 \text{ hours} \)
- Prolonged: > 24 hour, up to 30 days
- Permanent: > 30 days
<table>
<thead>
<tr>
<th>Nature of body contact</th>
<th>Contact duration</th>
<th>Biological effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cytotoxicity</td>
</tr>
<tr>
<td>A - limited</td>
<td>(≥ 24 h)</td>
<td>X</td>
</tr>
<tr>
<td>B - prolonged</td>
<td>(&gt; 24 h to 30 d)</td>
<td>X</td>
</tr>
<tr>
<td>C - permanent (&gt; 30 d)</td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

- **Surface device**
  - **Intact skin**
    - A: X
    - B: X
    - C: X
  - **Mucosal membrane**
    - A: X
    - B: X
    - C: X
  - **Breached or compromised surface**
    - A: X
    - B: X
    - C: X

- **External communicating device**
  - **Blood path, indirect**
    - A: X
    - B: X
    - C: X
  - **Tissue/bone/dentin**
    - A: X
    - B: X
    - C: X
  - **Circulating blood**
    - A: X
    - B: X
    - C: X

- **Implant device**
  - **Tissue/bone**
    - A: X
    - B: X
    - C: X
  - **Blood**
    - A: X
    - B: X
    - C: X

- **The crosses indicate data endpoints that can be necessary for a biological safety evaluation, based on a risk analysis.** Where existing data are adequate, additional testing is not required.
Biological Evaluation Tests

- Consider tests described in the table for the duration and nature of contact
- Also consider any additional specific areas of concern based on the product
- Consider the following for the biological tests
  - Testing shall be done on the **finished device**
  - Extracts of the device shall be prepared based on ISO10993 Part 12
  - Specific chemicals of concern and type of patient exposure to be considered
Biological Evaluation Process

» Biological Evaluation Tests (Table in Annex A)
  ▪ Cytotoxicity
  ▪ Sensitization
  ▪ Irritation
  ▪ Acute Systemic Toxicity
  ▪ Subacute/Subchronic Toxicity
  ▪ Genotoxicity
  ▪ Implantation
  ▪ Hemocompatibility

» Additional effects to consider
  ▪ Chronic toxicity, carcinogenicity, reproductive toxicity,
  ▪ Biodegradation, immunotoxicity, toxicokinetics, etc.
How to test

» Many tests based on extracting the device

- Standard vehicles used
  - Polar – saline, water, culture media (w/o serum)
  - Non-polar – vegetable oil, alcohol
  - Additional – alcohol/saline, DMSO, culture media (w/ serum)

- Standard ratio test/control article to vehicle
  - <0.5 mm thick = 120 cm²/20 mL
  - >0.5 mm thick = 60 cm²/20 mL
  - Irregular = 4 grams/20 mL
  - Elastomeric = 25 cm²/20 mL

- Time and temperature
  - 37°C for 72 hrs???, 37°C for 24 hrs (cytotoxicity only)
  - 50°C for 72 hrs
  - 70°C for 24 hrs
  - 121°C for 1 hr
How it is prepped

» Include only portion with possible patient contact
» Extraction ratio based on surface area if possible
» Entire device or representative portions included
» Device subdivided into small pieces
ISO 10993-10: Tests for irritation & sensitization

» Required for all patient contact devices
» Encompasses two categories of tests
  ▪ Sensitization
  ▪ Irritation

» Sensitization
  ▪ Allergenic response; immune-mediated (T-cells)

» Irritation
  ▪ Localized, non-specific inflammatory response
  ▪ Selection of test methods determined by intended use
  ▪ Many methods conducted in rabbits

» Current ISO position on in vitro methods?
ISO 10993-10: Tests for irritation & sensitization

» Sensitization test methods
  - Guinea pig maximization
  - Buehler closed patch (in guinea pigs)
  - Murine local lymph node assay
Sensitization – Maximization Method

- 15 Guinea pigs/extract (10 T, 5 C)
- Fur-clip shoulder area, bilateral intradermal injections

Induction I Phase

- Site A – 0.1 ml of emulsion of Freund’s complete adjuvant (CFA) & vehicle
- Site B – 0.1 ml of extract without adjuvant
- Site C - 0.1 ml of extract emulsified in FCA
Sensitization – Maximization Method

» Induction II Phase (7 days later)
  ▪ Pre-treat skin (SLS) (why?)
  ▪ Topical dose of extract or control to pretreated site
  ▪ Occlude for 48 hours

» Challenge Phase (14 days later)
  ▪ Topical dose to untreated skin
  ▪ Occlude for 24 hours
  ▪ Evaluate for erythema & edema daily for 2 days

» Rechallenge option
Sensitization – Closed Patch Method

Also know as the Buehler method

15 Guinea pigs/extract (10 T, 5 C)

All topical exposure

**Induction Phase** -

- Fur-clip shoulder area
- Induction Phase (9 inductions)
  - Apply 1” x 1” patches or 0.5 ml
  - Occlude for 6 hours
  - Score for irritation 24 hours later
  - Repeat 3x weekly for 3 weeks
Sensitization – Closed Patch Method

» Challenge Phase (14 days later)
  ▪ Apply material to untreated skin
  ▪ Occlude for 6 hours
  ▪ Evaluate for erythema & edema for 2 days

» Rechallenge option
Sensitization – Local Lymph Node Assay

» Approved by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program (NTP)

» Listed as an option in ISO 10993-10

» Used for several years, then not accepted by the FDA

» Currently accepted by the FDA – case by case basis
Sensitization – Local Lymph Node Assay

» 15 CBA/J mice per extract
  ▪ 5 test extract
  ▪ 5 negative control
  ▪ 5 positive control

» Aqueous and non-aqueous vehicles (saline, DMSO, PEG, AOO)

» Daily topical dose to the skin (back of the ear) for 3 consecutive days
Sensitization – Local Lymph Node Assay

» 2-day resting period
» IV injection of radio-labeled thymidine (3H)
» Collect lymph nodes 5 hours later
» Measure level of radioactivity
» Calculate Stimulation Index (SI)
Tests for irritation

» Intracutaneous Irritation Test

- Uses intradermal injections of saline and sesame oil extracts
- Inject extract in 5 sites per rabbit
- Evaluate erythema and edema daily for three days
- Appropriate for devices with
  - tissue contact beyond intact skin
  - Implants
  - externally communicating
  - compromised skin/mucous membranes contact
Tests for irritation

» Skin Irritation Test

- Test article itself is applied to skin of rabbits – article must be thin and pliable
- Skin can be intact or abraded
- Animals scored for erythema and edema through 72 hours
- Used for devices with intact skin or minimally compromised tissue
Tests for irritation

» Mucosal Irritation

- Three main methods
- Penile, vaginal, hamster cheek pouch (oral mucosa)
- Penile and vaginal (conducted in rabbits)
  - Use saline and sesame oil extracts
  - Repeated dose followed by macroscopic observations
  - Final microscopic evaluation conducted on H&E stained sections of tissue
  - Selection of method dependent on mucosal contact and use of the device
Acute systemic toxicity

- Systemic effects from a single dose
- Saline and sesame oil extracts
  - Saline dosed IV, sesame oil IP
- Mortality, clinical signs, weight loss evaluated over 72 hours
- Often performed as an in vivo screen
- Other extraction vehicles can be used or liquid samples injected themselves
ISO 10993-11: Tests for systemic toxicity

» Subacute/Subchronic/Chronic Toxicity Studies

- 4, 13, or 26 weeks in rats (both sexes)
- Test and control articles implanted (typically SC)
- Dose to exaggerate the clinical exposure
- Body weights, clinical observations, hematology, clinical chemistry, organ weights, histopathology of implant sites and organs
ISO 10993-6: Implantation

- Difficult to address with material characterization or in vitro assays
- Implantation evaluates a materials potential effects on surrounding living tissue (muscle, bone, subcutaneous, etc)
- Can be conducted in a variety of species and sites
  Commonly conducted in rabbits
- Size of the implant may determine species
- While ISO 10993-6 primarily addresses local tissue “biocompatibility”, functional and performance effects frequently required animal models.
Implantation – Local Effects

» Multiple evaluation intervals
  ▪ Short Term, 2 to 12 weeks
  ▪ Long Term, > 12 weeks
  ▪ Degradable materials – last interval when material is fully absorbed by the body

» Evaluation
  ▪ Macroscopic and microscopic scoring of implant sites
  ▪ Tests sites are compared to control article; HDPE (high density polyethylene) for example
ISO 10993-4: Selection of tests for interaction with blood

» Testing in this category may not be required, but any device with bone contact, also will have blood contact.

» Testing requirements depend on the nature of the device and blood contact.

» Five main areas that may be considered:
  • Thrombosis
  • Coagulation
  • Platelets
  • Hematology
  • Complement

» Hemolysis is the only well-defined assay and only test likely to be needed for an orthopedic device.

» Most are addressed in vitro except thrombosis.
Since surface geometry and material characteristics affect the response, testing requires the actual device vs. extracts.

Wide variation in size, location of use, duration of exposure, along with other factors, make having a single, standard test method for all less practical.

Due to device sizes, large animal models are typically needed:
- Canine – sites, pros & cons
- Swine – sites, pros & cons
- Sheep – sites, pros & cons

Mimic clinical use as much as possible:
- Type vessel
- Vessel diameter
- Duration
- Anti-coagulant therapy
- Method of placement
Thrombosis

At the end of the implant period, vessel with device is placed is removed and scored for thrombus formation.
ISO 10993-3: Tests for genotoxicity

» Test battery approach
  ▪ All can be *in vitro* (ISO)
  ▪ Can have one non-mammalian cell assay
  ▪ For mammalian cell assay - either two different assays or one with 2 endpoints
  ▪ Examples:
    ● bacterial assay for gene mutations – Ames = ~short duration
    ● test for gene mutations in mammalian cells – Mouse lymphoma - longer
    ● test for clastogenicity in mammalian cells – Chromosomal aberration

» ISO - only conduct in vivo assay if positive response

» FDA officially has not adopted Part 3
  ▪ Follows ICH guidelines ???
  ▪ Three test battery, but one in vivo assay