# Validation Guide Summary

## C-Flex® 374

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# Validation Guide Summary: C-Flex® 374

## 1.0 Summary

C-Flex® 374 is an animal derived component-free tubing. It has exceptional tensile and tear strength, is biocompatible and has excellent chemical resistance. C-Flex® 374 is also heat-sealable and weldable. It has low protein binding, an ultra-smooth inner bore and is transparent. It is temperature stable from -67 °C to 135°C (-85°F to 275°F) and can be sterilized by autoclave, ethylene oxide or gamma irradiation.

C-Flex® 374 resin meets USP Class VI requirements. C-Flex® 374 tubing is manufactured according to GMP.

## 2.0 Typical Physical Properties

| Property | ASTM Standard | C-Flex® 374 |
|---|---|---|
| Durometer hardness (Shore A) | ASTM D2240 | 60 |
| Tensile Strength (psi) | ASTM D412 | 1190 |
| Ultimate Elongation (%) | ASTM D412 | 915 |
| Temperature Range (°C/°F) | | -67°C to 135°C<br>-85°F to 275°F |

## 3.0 Summary of Results

| Method Description | Reference Standard | Result |
|---|---|---|
| Ames Genotoxicity | ISO 10993-3 | Passed |
| Hemolysis – Indirect Contact | ISO 10993-4 | Passed |
| Systemic Toxicity - Rabbit Pyrogen (Material Mediated) | ISO 10993-11 | Passed |
| Sterility Test: Bacteriostasis & Fungistasis | USP <71> | Passed |
| LAL Gel Clot Endotoxin | USP <85> | Passed |
| Biological Reactivity Tests, In Vitro | ISO 10993-5 | Passed |
| Class VI Plastics - Biologic Reactivity Tests, *In Vivo*: Post-Gamma Irradiation Samples | USP <88> | Passed |
| Physicochemical Tests for Plastics | USP <661> | Passed |

| | | |
|---|---|---|
| Rubber Closures for Containers | EP 3.2.9 | Passed |
| Physicochemical Testing with an Alternative Extract | See Summary Section, 5m. | |

## 4.0 Chemical Compatibility

| | |
|---|---|
| **Weak Acids** | Acceptable |
| **Strong Acids** | Acceptable |
| **Weak Bases** | Acceptable |
| **Strong Bases** | Acceptable |
| **Salts** | Acceptable |
| **Alcohols** | Not recommended |
| **Organic Solvents** | Not recommended |
| **Oil/Water emulsions** | Test before using |

## 5.0 Biocompatibility, Physicochemical & Extractable Testing

### 5a. ISO 10993-3 Ames Genotoxicity Test

Genotoxicity tests are conducted to detect compounds that could potentially cause genetic damage. Genotoxic compounds can potentially cause cancer or heritable defects in humans. The Ames Genotoxicity Test assesses the ability of potentially genotoxic compounds to reverse genetic mutations in specific reference bacteria, and has been shown to predict potential carcinogenicity and mutagenesis in humans.

Test: A sample of C-Flex® 374 was tested by Toxikon Corporation in accordance with ISO 10993-3:2003, Biological evaluation of medical devices - Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity.

The test article was extracted with USP 0.9% Sodium Chloride for Injection (NaCl) and Dimethyl Sulfoxide (DMSO) for 24hrs at 70°C. Triplicate cultures of four strains of histidine deficient ($his^-$) *Salmonella typhimurium* and one strain of tryptophan deficient ($tryp^-$) *Escherichia coli* were exposed to the resulting extractant. Triplicate cultures of these strains were also exposed to NaCl and DMSO as negative controls. As positive controls, cultures of these strains were also exposed to strain-specific known mutagens (Sodium Azide, 2-Nitrofluorene, 9-Aminoacridine, 4-Nitroquinoline 1-Oxide, 2-Aminoanthracene) on triplicate plates.

Results: The mutant strains exposed to the test article extract did not exhibit a statistically significant number of revertant colonies relative to those exposed to the negative controls. The positive controls demonstrated statistically

significant growth in response to exposure to known mutagens, confirming that the test was valid. The test article was therefore deemed non-mutagenic in the test species.

**5b. ISO 10993-4 Hemolysis Indirect Contact**

The Hemolysis test assesses the potential for indirect contact of a given sample with blood to cause the rupture of erythrocytes (red blood cells).

Test: A sample of C-Flex® 374 was tested by Toxikon Corporation in accordance with ISO 10993-4, 2002 Biological Evaluation of Medical Devices – Part 4: Selection of Tests for Interactions with Blood..

The test article was added to a test vial containing USP 0.9% Sodium Chloride for Injection. The test article positive control and negative control were prepared in triplicate. All tubes were incubated in a 37°C±2°C water bath for 30 minutes. The rabbit blood was collected in tubes containing an anticoagulant and diluted in NaCl. After the incubation period, 0.2mL of fresh diluted rabbit blood was added to all vials. All vials were incubated at 37°C±2°C for an additional 0 minutes. After incubation, the vials were centrifuged. The absorbance of each supernatant was determined spectrophotometrically at 545 nm. The percent hemolysis of the test article was determined.

Results: The percent hemolysis resulting from indirect contact of the product with rabbit blood was 0.6%. The test article is considered non-hemolytic.

**5c. ISO 10993-11 Tests for Systemic Toxicity – Rabbit Pyrogen Test (Material Mediated)**

The rabbit pyrogen test is performed to qualitatively determine whether a given test article contains pyrogens. Pyrogens can provoke a significant febrile reaction in a human patient who receives a parenteral drug product that has come into contact with a contaminated test article.

Test: A sample of C-Flex® 374 was tested by Toxikon Corporation in accordance with USP32, NF 27, 2009; <151> Pyrogen Test and ISO 10993-11:2006 Biological Evaluation of Medical Devices Part 11, Tests for Systemic Toxicity.

The test article was immersed in USP 0.9% Sodium Chloride for Injection (NaCl) at 70°C for 24hrs. The resulting extract was administered to test subjects (rabbits) via IV injection at a dose of 10 mL per kg of body mass. A control rabbit was similarly injected with the control sample (NaCl). The body temperatures of the animals were measured 30 minutes prior to injection and again every 30 minutes between the 1 hour and 3 hours marks post-injection.

Results: The three test rabbits showed a rise of 0.2°C, 0.3°C and 0.3°C in body temperature post-injection. There was a 0.2°C change in body temperature in the control rabbit post-injection. A test article is considered pyrogen-free provided

that none of the test subjects display an increase in body temperature of more than 0.5°C post-injection, therefore the test articles were deemed pyrogen-free.

**5d. USP <71> Sterility Tests: Bacteriostasis & Fungistasis**

Bacteriostasis and Fungistasis testing is performed to determine whether or not a given test article has the potential to inhibit the growth of bacteria and/or fungi, respectively, and therefore potentially interfere with standard Sterility Tests.

Test: Samples of C-Flex® 374 were tested by NAMSA in accordance with USP <71>: Sterility Tests.

Test articles were placed in three culture vessels containing Soybean Casein Digest Broth (SCDB) and three vessels containing Fluid Thioglycollate Media (FTM). Corresponding positive controls were prepared using the same media in culture vessels that did not contain samples of test article. The product-containing and positive control flasks were inoculated with *Bacillus subtilis* (SCDB), *Clostridium sporogenes* (FTM), *Candida albicans* (SCDB), *Aspergillus brasiliensis* (SCDB) and *Staphylococcus aureus* (FTM) *Pseudomonas aeruginosa* (FTM). Flasks containing SCDB were incubated at 20°C - 25°C and flasks containing FTM were incubated at 30°C - 35°C until positive for microbial growth or until five days had passed.

Results: All test article-containing flasks and positive control flasks were positive for microbial growth within three days. The test article was therefore deemed non-bacteriostatic and non-fungistatic.

**5e. USP <85> LAL Gel Clot Endotoxin**

Endotoxins are lipopolysaccharide complexes found in Gram negative bacterial cell walls. They can cause significant illness in humans. The Limulus Amebocyte Lysate (LAL) Gel Clot Test is used to detect and quantify endotoxin levels in test samples.

Test: Samples of C-Flex® 374 were tested by Toxikon Corporation in accordance with USP 27, NF 22, 2004, <85> Bacterial Endotoxins Test; Guidelines of Validation of the Limulus Amebocyte Lysate Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological products and Medical Devices, December 1987.

The test articles were immersed in sterile Water for Injection (WFI) at room temperature for 60 minutes. The extracts were pooled and tested in duplicate. A positive control was prepared in duplicate using serial dilutions of endotoxin standard. A positive product control was prepared using the test article extract and the endotoxin standard. Sterile WFI was used as a negative control. LAL was added to all test and control samples, which were then incubated at 37°C for 60 minutes.

Results: Both the positive control and positive product controls demonstrated agglutination indicating that the product did not interfere with the test. Neither the negative control samples nor the product test samples showed agglutination. The endotoxin level was therefore determined to be below the limit of detection for this assay, i.e. <0.06 EU/ml and <2.4 EU/device. The test articles were certified endotoxin-free.

**5f. ISO 10993-5, 1999 Biological Reactivity Tests, *In Vitro***

Cytotoxicity testing assesses the potential of a given material to have a toxic effect on living cells.

Test: Samples of C-Flex® 374 were tested by Toxikon Corporation in accordance with ISO 10993-5, 1999; <87> Biological Reactivity Tests, *In Vitro*.

Test samples were immersed in Serum-Supplemented Minimum Essential Medium at 37°C for 24hrs. Positive control (Natural Rubber) and negative control (Negative Control Plastic) samples were also extracted as above. Duplicates of all three extracts were incubated with L929 mouse fibroblast cells at 37°C for 48hrs. Cultures were monitored for cellular degeneration and malformation and rated on a scale of 0 (No Biological Reactivity) to 4 (Severe Biological Reactivity).

Results: The test article samples and the negative controls scored a Grade 0 for Biological Reactivity after 48hrs. The positive controls scored a Grade 4 at the 48hr mark. Samples are deemed to meet the test requirements if they exhibit a Biological Reactivity of no more than Grade 2 (Mild Reactivity). The test articles are therefore considered non-cytotoxic.

**5g. USP <88> Biological Reactivity Tests, *In Vivo*:**

The USP Class VI Plastics Test assesses the potential toxicity of a given test article by introducing a sample into live animals systemically, intracutaneously and through implantation. Test animals are then monitored for signs of irritation and/or toxicity.

Test: Samples of C-Flex® 374 were tested by Toxikon Corporation in accordance with USP <88> Biological Reactivity Tests, *In Vivo*.

Test articles that had been gamma irradiated at 40.61 - 47.04 kGy were immersed in USP 0.9% Sodium Chloride (NaCl), Cottonseed Oil (CSO), 1 in 20 Ethanol in NaCl (EtOH) or Polyethylene Glycol 400 (PEG) at 70°C for 24hrs. The test article extracts and corresponding controls (samples of each extractant that had not been exposed to the test article) were injected systemically into mice and intracutaneously into rabbits and the animals were observed for 72 hours for signs of skin reactivity or toxicity. In addition, the test article was implanted into the paravertebral muscles of rabbits, which were then observed for 7 days for macroscopic signs of hemorrhage, necrosis, discoloration, encapsulation and/or infection.

Results: None of the animals injected systemically with test article extracts or controls exhibited any signs of toxicity. Similarly, none of the animals injected intracutaneously with test article extracts or controls exhibited any signs of erythema, edema or clinical toxicity. Further, none of the implanted animals exhibited any signs of toxicity at the implantation sites relative to the control sites. The test article therefore met the requirements of the USP Class VI Test for Biocompatibility.

**5h. USP <661> Physicochemical Tests for Plastics**

Physicochemical testing is performed to assess the suitability of the test article for use in contact with drug products for parenteral administration in humans.

Test: A sample of C-Flex® 374 was tested by NAMSA in accordance with USP <661> Containers, Physicochemical Tests – Plastics.

The test article was immersed in USP Purified Water for 70°C for 24hrs. The extract was then tested for Non-Volatile Residue, Residue on Ignition, Heavy Metals as Lead and Buffering Capacity per USP <661>.

Results: The test results are summarized in the table below.

| Assay | Assay Results | Limits Based on Area |
|---|---|---|
| Non-Volatile Residue | <1 mg | ≤ 15 mg |
| Residue on Ignition | <1 mg | ≤ 5 mg |
| Heavy Metals | < 1 ppm | ≤ 1 ppm |
| Buffering Capacity | <1 ml | ≤ 10.0 ml |

The test article sample met the criteria established per USP <661> for all of the tests performed, as shown above.

**5i. EP 3.2.9 Testing of Rubber Closures for Containers**

The European Pharmacopoeia Monograph Testing Section 3.2.9 assesses the physical and physicochemical characteristics of rubber closures intended for use with containers that will house aqueous preparations for parenteral use.

Test: Samples of C-Flex® 374 were tested by NAMSA in accordance with EP 3.2.9.
The test articles were examined for Identity (via Elasticity, Infrared Analysis and Total Ash Content), Appearance, Acidity, Absorbance, Reducing Substances, Heavy Metals, Zinc, Ammonium, Residue on Evaporation and Volatile Sulphides. Test results were assessed relative to the specified EP limit for each characteristic.

Results: The test article met the EP limit for each characteristic, with the exception of Identity via IR Analysis and Total Ash Content. The EP standard requires a comparison of the test article result to results obtained using an appropriate "type" sample. No "type" sample was used, therefore the results of those tests did confirm the Identity of the test sample but did not technically meet the EP limit as none could be established with the appropriate "type" sample.

**5j. Physicochemical Testing with an Alternative Extract**

Physicochemical testing with an alternative extract is performed to assess the levels of lipophilic extractables in polymeric samples. Isopropyl Alcohol (IPA) is used as the extractant in these tests. Formal limits for IPA-soluble extractables have not yet been established, but in general lower levels of lipophilic extractables are preferred for materials that will come into contact with blood or tissues.

Test: A sample of C-Flex® 374 was tested by NAMSA in accordance with NAMSA's standard protocol for Physicochemical Testing with an Alternative Extract.

The test article was immersed in (IPA) at 70°C for 24hrs. The extract was assessed for Non-Volatile Residue, Residue on Ignition and Turbidity. The appearance of the extract on visual inspection was compared to a control sample of IPA.

Results: The results are summarized below.

Non-Volatile Residue - 1826 mg
Residue on Ignition - <1 mg
Turbidity – 698 NTU
Appearance of Test Article Extract - white and cloudy
Appearance of IPA Control - clear and colorless

**5k. Extractable Data (Saint-Gobain Protocol)**

The Saint-Gobain extractables study approach to performing a comprehensive extractables analysis is described in the Extractable Test Strategy (found at the link below) and uses a solvent selection following the BPOG (BioPhorum Operations Group) protocol and USP <665> as a guide. The Saint-Gobain Extractables protocol is also available from our website.

Saint-Gobain publishes its extractable data using the BPOG Extractable Report template. Data is available as an Excel spreadsheet and PDF document in alignment with the recommendations from the BPOG Supplier Partner Phorum to facilitate end user safety assessments. End users can gain access to the raw data in Excel spreadsheets and the approved PDF version of the Extractables Report by following the link below.

https://www.biopharm.saint-gobain.com/extractables-information

NOTE: The supplier warrants that the information is correct at point of download, but the end user must acknowledge that the controlled report is the verified document and is accountable for any errors introduced during manipulation of the excel data.

# Validation Guide Summary

## C-Flex® 374

**Approval Signatures**

| Role | Name | Title | Signature and Date |
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**Revision History**

| Revision | Revision Date | Section(s) Affected | Revision Description |
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| 0 | 08Oct2013 | All | Original Issue |
| 1 | 08Oct2015 | Table of Contents, 3.0, 6.0 | Removed Regulatory Information Overview statement. Added technical summary. Added revisions section. |
| 2 | 08Mar2016 | Table of Contents, 4.0, 6.0 | Revised technical summary listing. Adjusted page numbers. Combined 4d and 4e into Manufacturing Standard for both facilities. Revisions to Addendum page. |
| 3 | 12Sep2016 | Table of Contents, 5n, 6.0 | Added Chemical Identification and Semi-Quantification of Extractables. Revisions to Addendum page. |
| 4 | 08Dec2020 | All | Validation Guide updated to reflect current design template. Product Stewardship and DMF information removed. Physical properties table updated. Elastomeric Closures, Total Organic Carbon Analysis and Chemical Identification and Semi-Quantification of Extractables section replaced with link to updated Extractables data available for download. |

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