Validation guide summary

Accusil Platinum-cured silicone tubing
1. Introduction
Flexicon’s Accusil™ platinum-cured silicone tubing is manufactured from a USP class VI raw material in an ISO 14644-1 Class 7 cleanroom operating within an ISO 9001 quality system. Accusil is compliant with a list of compendial testing as summarised in Section 5A. Accusil platinum-tubing has been exclusively designed for use in Flexicon peristaltic technology. When used together Accusil exhibits excellent dispensing accuracy of up to +/- 0.5%.

Accusil key features:
- Laser Traceability: 100% traceability with laser etched lot number, product specification and use by date
- Cleanroom manufactured in an ISO 14644-1 Class 7 environment
- Double bagged for protection against contamination
- Fully post cured
- Suitable for gamma irradiation up to 50kGy

Flexicon founded in 1986 in Denmark, is a company that specialises in the development and manufacture of aseptic filling systems for the biotechnology and pharmaceutical market with an emphasis on precision, efficiency and flexibility.

Watson-Marlow Fluid Technology Group (WMFTG) is the world leader in niche peristaltic and sinusoidal pumps and associated fluid path technologies. Founded on nearly 60 years of supplying engineering and process expertise and with over one million pumps installed worldwide, our pumps are tried, tested and proven to deliver. Flexicon has been a part of WMFTG since 2008.

Watson-Marlow is a wholly owned subsidiary of Spirax-Sarco Engineering plc. Spirax-Sarco’s headquarters are in Cheltenham, England and is listed on the London Stock Exchange.

2. Conditions of use
Accusil tubing may be sterilised using either of the following methods:
- Gamma irradiated to 50kGy
- Autoclaved at 121°C for up to 60 minutes

2a Working temperature and pressure rating
The working temperature range of Flexicon Accusil™ silicone tubing is –20°C to 80°C (–4°F to 176°F).

3. Chemical compatibility
A general guide on chemical compatibility of Accusil tubing can be found on Watson-Marlow Fluid Technologies Group website wmf tg.com/chemical

4. Materials, manufacturing and regulatory compliance statements

4a Materials of construction
Accusil is made of polydimethylsiloxane (PDMS)

4b Manufacturing environment
Accusil is manufactured according to the principles of GMP in an ISO 14644-1 class 7 cleanroom within a facility operating an ISO 9001 quality management system.

4c Country of origin
Accusil is manufactured in Falmouth, Cornwall, United Kingdom.

Watson-Marlow Fluid Technology Group comprises ten established brands, each with their own area of expertise, but together offering our customers an unrivalled breadth of solutions for their filling and dispensing applications.
4d **Compliance declaration summary**

Table 1 details the different substances that are not present in the raw material, manufacturing process or final composition of Accusil.

For full compliance statements please refer to the compliance summary sheet available from the website available on request.

Table 1: List of compliance statements for Accusil and substances not found in the processing of or raw materials for Accusil

<table>
<thead>
<tr>
<th>Substances not present/Compliance statement</th>
<th>Raw material</th>
<th>Manufacturing process</th>
<th>Final product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerin</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Animal Derived Content</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Melamine</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phthalates</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bisphenol A (BPA)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Latex</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*: denotes not present or not added

4e **REACH legislation**

All raw materials, compounds used in the manufacturing process and the final Accusil product comply with the REACH regulation. None of the chemicals used in the manufacture of Accusil are on the candidate list of substances of 2008 or the list of Substance of Very High Concern (SVHC).

4f **RoHS**

In compliance with the restriction of hazardous substances (RoHS) directives, no listed substances are used in the manufacture of Accusil.

4g **Storage conditions**

To maintain the performance of the tubing throughout its life, tubing should be stored in a cool, dry environment away from direct sunlight. The normal storage temperature is between –10°C to 40°C.

Wherever possible, original packaging should be maintained. Stock should be rotated on a first in, first out (FIFO) basis. The performance of any tubing beyond its use by date, or which has not been stored according to the recommendations outlined above, cannot be assured.

5. **Biocompatibility and physiochemical testing**

5a **Summary table**

Table 2 contains a summary of all the compendial and non-compendial testing performed. Full test methods and results are available on request. All tests were carried out by third party contract labs.

<table>
<thead>
<tr>
<th>Test reference</th>
<th>Test Description</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>USP &lt;87&gt;</td>
<td>Biological reactivity test, In Vitro</td>
<td>PASS</td>
</tr>
<tr>
<td>USP &lt;88&gt;</td>
<td>Biological reactivity test, In Vivo</td>
<td>PASS</td>
</tr>
<tr>
<td>ISO 10993-4</td>
<td>Haemolysis Test—Avian method</td>
<td>PASS</td>
</tr>
<tr>
<td>ISO 10993-5</td>
<td>Biological evaluation of medical devices, tests for In Vitro cytotoxicity</td>
<td>PASS</td>
</tr>
<tr>
<td>ISO 10993-6</td>
<td>Biological evaluation of medical devices, implantation</td>
<td>PASS</td>
</tr>
<tr>
<td>ISO 10993-10</td>
<td>Biological evaluation of medical devices, irritation</td>
<td>PASS</td>
</tr>
<tr>
<td>ISO 10993-11</td>
<td>Biological evaluation of medical devices, systemic toxicity</td>
<td>PASS</td>
</tr>
<tr>
<td>ISO 10993-10</td>
<td>Kligman maximisation—test for irritation and delayed type hypersensitivity</td>
<td>PASS</td>
</tr>
<tr>
<td>USP &lt;85&gt;</td>
<td>Limulus Amboecyte Lysate (LAL) bacterial endotoxin assay</td>
<td>REPORT</td>
</tr>
<tr>
<td>USP &lt;381&gt;</td>
<td>Physicochemical tests on elastomeric closure materials</td>
<td>PASS</td>
</tr>
<tr>
<td>E.P.3.1.9</td>
<td>European Pharmacopoeia 3.1.9 silicone elastomer for closures and tubing</td>
<td>PASS</td>
</tr>
</tbody>
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<td>Biological evaluation of medical devices—tests for In Vitro cytotoxicity</td>
<td>PASS</td>
</tr>
</tbody>
</table>

5b **USP <87> Biological reactivity tests, In Vitro, post sterilisation samples**

Samples of Accusil were gamma irradiated at 45–55kGy and tested in accordance with USP32, NF 27, <87>, Biological reactivity tests, In Vitro. The biological reactivity of a mammalian monolayer, L929 mouse fibroblast cell culture in response to Accusil was determined. Samples of Accusil tubing, positive control (rubber) and negative control articles were prepared at 37°C for 48 hours. Biological reactivity was rated on a scale ranging from Grade 0 (no reactivity) to Grade 4 (severe reactivity).

Results: No reactivity (grade 0) was exhibited by the cell cultures when exposed to Accusil. Therefore Accusil is not cytotoxic and passed the requirements of USP <87> biological reactivity tests.

5c **USP <88> Biological reactivity tests, In Vivo, post sterilisation samples**

USP Class VI Plastics Test assesses the potential toxicity of a given test article by introducing a sample in Vivo systemically, intracutaneously and through implantation.

Samples of Accusil were tested in accordance with USP32, NF 27, <88>, Biological reactivity tests, In Vivo. This included the immersion of the test articles in the following solutions: USP 0.9% sodium chloride, cottonseed oil, 1 in 20 Ethanol in NaCl and Polyethylene glycol 400 at 70°C for 24 hours.

Results: Accusil extracts and implants showed no toxicity, therefore Accusil met the requirements of USP <88> Class VI biological reactivity tests.

5d **ISO 10993-4 Haemolysis**

The haemolysis test assesses the potential for haematological effects as a result of a single dose systemic injection. Samples of Accusil were tested in accordance with ISO 10993-4, 2002, Biological Evaluation of Medical Devices—Part 4: Selection of tests for interactions with blood.

Results: Accusil showed no signs of haemolytic activity. Therefore Accusil passed the requirements of ISO 10993-4.

5e **ISO 10993-5 Biological evaluation of medical devices—tests for In Vitro cytotoxicity**

The biological reactivity of a cell culture, in response to extracts from Accusil was determined. The maintenance medium on the cell cultures was replaced by extracts of Accusil, or control article.

The cell cultures were incubated for 48 hours at 37°C ±1°C. Biological reactivity was evaluated by a photo spectrometer at 450nm wavelength.

Results: Accusil showed no signs of cytotoxic activity. Therefore Accusil passed the requirements of ISO 10993-5.

5f **ISO 10993-6 Biological evaluation of medical devices, implantation**

The purpose of this test is to evaluate the solid material in direct contact with living tissue.

Accusil was tested in accordance with ISO 10993-6. Strips of Accusil (1mm x 1mm x 10mm) and standard negative control plastics were evaluated. The test sites were examined for inflammation, encapsulation, necrosis, haemorrhage and discolouration macroscopically.

Results: Accusil did not demonstrate any difference when compared to the control implant sites. Therefore Accusil passed the requirements for ISO 10993-6.

5g **ISO 10993-10 Biological evaluation of medical devices, irritation**

The intracutaneous test is designed to evaluate local responses to the extracts of Accusil following intracutaneous injection. Accusil was tested in accordance with the requirements of ISO 10993-10. Accusil tubing is extracted using 0.9% sodium chloride for injection and cottonseed oil at 70°C for 24 hours.

Results: Accusil meet the requirements of ISO 10993-11 guidelines for the systemic injection test.

5h **ISO 10993-11 Biological evaluation of medical devices, systemic toxicity**

The systemic injection study is designed to screen Accusil tubing extracts for potential toxic effects as a result of a single dose systemic injection.
Accusil was tested in accordance with the requirements of ISO 10993-11. Accusil tubing was extracted using 0.9% sodium chloride for injection and Cottonseed oil at 70°C for 24 hours. The extracts were injected intradermally. After two weeks, an additional topical application was introduced to the site of intradermal injections. Results: Accusil meet the requirements of ISO 10993-11 guidelines for the systemic injection test.

5.1 ISO 10993-10 Kligman Maximisation Test

The purpose of this test is to detect the allergenic potential of a test article. Accusil was tested in accordance with ISO 10993-10. Samples of Accusil were extracted in USP 0.9% Sodium chloride for injection and Cottonseed oil at 70°C for 24 hrs. The extracts were injected intradermally. After two weeks, an additional topical application was introduced to the site of intradermal injections. Results: The skin sites that were exposed to the test articles and negative control showed no signs of erythema or edema. Therefore Accusil is deemed not to contain any allergic potential.

5.2 USP <381> Physicochemical tests on elastomeric closure material

Extracts of Accusil were prepared according to the requirements of USP 32, NF 27, Chapter 381 as directed under Physicochemical tests. The results of the tests are summarised in Table 3 below.

Results: Based on the evaluation criteria mentioned above, Accusil tubing meets the requirements of the USP <381> section physicochemical tests.

5.3 European Pharmacopoeia 3.1.9

Extracts of Accusil were prepared in accordance with the requirements of European pharmacopoeia, 6.8, Chapter 3.1.9 Silicone elastomer for closures and tubing. The results of test are summarised in Table 4 on the following page.

Results: Based on the results of the tests, Accusil tubing meets the requirements of EP 3.1.9 section Physicochemical tests.

5.4 USP <85> Limulus amebocyte lysate (LAL) bacterial endotoxin assay

Endotoxins are lipopolysaccharide complexes in gram negative bacterial cell walls. The limulus amebocyte lysate (LAL) gel clot test is used to detect and quantify endotoxin levels in test samples. Accusil was tested in accordance to the requirements of USP 85. Accusil (100 cm²) was extracted in 50mL of LAL reagent water at room temperature for 60 minutes. The Accusil extract was assayed in duplicate at the undiluted concentration. A positive control was prepared using serial dilution of the endotoxin standard. A product sample was prepared from the Accusil extract and the endotoxin standard. LAL was added to the samples, which were incubated at 37°C for 10 minutes.

Results: Accusil extracts had an EU/ml value of 0.0174 which is less than the value of 0.25 EU/ml stated for water for injection.

6. Extractables

Sections of Accusil tubing were subjected to extraction in multiple solvents at controlled temperatures. The test material was extracted in a 6cm²:1mL surface area to volume ratio. The solvent extracts were then analysed using high pressure liquid chromatography-diode array detector-mass spectrometry (HPLC-DAD/MS), headspace gas chromatography mass spectrometry (HS-GC/MS), total ionic chromatography mass spectrometry (TIC-MS) direct injection gas chromatography mass spectrometry (DI-GC/MS) and inductively coupled plasma-mass spectrometry (ICP/MS). HPLC-DAD/MS is used to detect the presence of non-volatile and UV active extractables. DI-GC/MS can be used to identify if there are any semi volatile compounds present in the extracts whilst volatile extractables can be detected using HS-GC/MS. Potential elemental impurities can be identified by ICP-MS.

Results: These studies have shown the extractables are indicative of the materials of construction. WMFTG can provide assistance in the evaluation of extractables data for risk assessment purposes.

7. Conclusions

Accusil has passed a number of pharmacopoeial and ISO testing, a summary of the results are disclosed within. For further information with full compliance statements and test reports, please fill in a request form available on the WMFTG website.

Compliance summary & full validation guide for Accusil are available on the wmftg.com website: wmftg.com/accusil-validate
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