Pre-processing Testing Specification on Sugi® Material

Tests/ items	Nominal values
Composition	
Cotton	Approx. 35 %
Regenerated cellulose	Approx. 65 %
Latex / DEHP	None
CMR/SVHC	None
Substances of human/animal origin	None
Nanomaterial according to MDR §2 (18-21)	None
Appearance/ Description	
Compressed white-cream absorbent sponge material	
Purity testing	
According to DAB or EP „Verbandswatte aus Baumwolle und Viskose" in the current version.	
Surfactants	Foam must not completely cover the surface after 5 minutes
Water soluble substances	Max. 0.6%
Sulphide	Test solution must be coloured weaker than the reference solution
Drying loss	Max. 8%
Sulphate residue test (sulphate ash)	Max. 0.3%
Fluorescence	Light brown fluorescence and some yellow particles, however must not show strong blue, except for individual fibres.
Foreign fibres	none
Technical properties	
pH value of test solution	6,0 – 7,5
Water absorption	> 1200 %
Area weight range	Approx. 120 – 670g/m²
Bioburden	
Bioburden limit for absorbent materials	< 200 cfu /5 g
Sterilization	
Ethylene oxide	Sterilization by ethylene oxide is recommended for Sugi Products. One-time re-sterilization with ETO does not impair the characteristics of the product.
Gamma rays	One-time re-sterilization with gamma rays will lead to a reduction of the absorptive capacity
Storage / Shelf life	
In sealed climate-secure foils at room temperature (5 °C – 30 °C). Unsealed storage in a humid environment will lead to an increase of the dry layer thickness (absorption of humidity). Shelf life is 5 years / 60 months.	

ADDRESS
QUESTALPHA GmbH & Co. KG
Im Heerfeld 7
35713 Eschenburg
Deutschland/Germany

CONTACT
+49 (0) 2774 705 505
info@questalpha.com
www.questalpha.com

REGISTER/VAT
Amtsgericht Wetzlar
HRA 7726
VAT Number:
DE326299428

BANK DETAILS
Commerzbank AG
IBAN: DE42 5164 0043 0169 2540 00
BIC: COBADEFFXXX

GENERAL PARTNER
QUESTALPHA Verwaltungs-GmbH, Eschenburg
Amtsgericht Wetzlar HRB 7958

CHAIRMAN
Jens T.W. Kuhn

MANAGING DIRECTOR
Dr. Sebastian Breuer

Biological Tests on Sugi® material

Cytotoxicity (Project 91111501, 1991; 97z021, 1997)

Method:	**ISO 10993-5** Biological evaluation of medical devices, Tests for *in vitro* Cytotoxicity.
Test material:	SUGI® strips Dimension: 35 x 7.5 x 1.5 mm
Extract production:	Extraction medium: phosphate buffered physiological saline solution, pH 7.4. Temperature and duration: 37 °C, 24 hours (1991) 37 °C, 72 hours (1997)
Cell culture:	L 929 mouse fibroblasts
Controls:	Positive: Dilution series Negative: Extraction medium
Test procedure:	6 parallel cultures per tested dilution of the extracts (100, 60, 30, 10, 3, 1 and 0.3 %). Dyed with crystal violet. Calculation of cell growth inhibition from the extinction values at 580 nm
Results:	Test sample and negative control showed no signs of reactivity (Grade 0) with 30 % concentration of dilution
Conclusion:	SUGI® (non-sterilized and sterilized) does not exhibit pronounced cytotoxicity

Sensitization (Report: 10-05-0923/00-92, 1992)

Method:	**ISO 10993-10** Biological evaluation of medical devices, Tests for irritation and skin sensitization.
Test Material:	SUGI® strips Dimension: 35 x 7.5 x 1.5 mm
Extract production:	Extraction medium: phosphate buffered 0.9% saline solution, pH 7.4. Temperature and duration: 37 °C, 72 hours
Test population:	20 guinea pigs
Control population:	10 guinea pigs
Test procedure:	Induction exposure to undiluted extract of test material. After Extract production with surface to volume ratio 3 cm^2/ml. Release 14 days afterwards, with this extract
Results:	After 24, 48 and 72 hours no allergic reaction, no toxic effects. Classification numbers for erythema and oedema = 0.
Conclusion:	SUGI® is non-sensitizing

Irritation – Skin (Report: 10-03-0922/01-92, 1992)

Method:	**ISO 10993-10** Biological evaluation of medical devices, Tests for irritation and skin sensitization.
Test material:	Undiluted extract made of SUGI® strips
Test population:	3 rabbits
Test procedure:	duration of contact: 4 hours
Results:	After 30 minutes, 1, 24, 48 and 72 hours., no allergic reaction, no toxic effects. Irritation index for animals = 0.
Conclusion:	SUGI® is non-irritating to the skin

Irritation – Ocular (Report: 10-03-0937/00-92, 1992)

Method:	**ISO 10993-10** Biological evaluation of medical devices, Tests for irritation and skin sensitization.
Test material:	Undiluted extract made of SUGI® strips
Test population:	3 rabbits
Test procedure:	duration of contact: 4 hours
Results:	After 1, 24, 48 and 72 hours, no allergic reactions, no toxic effects or eye lesions. Classification number = 0.
Conclusion:	SUGI® is non-irritating to the ocular surface

Update biocompatibility

Update 2014

Cytotoxicity (Project 14Z057, 2014)

Method:	**ISO 10993-5** Biological evaluation of medical devices, Tests for *in vitro* Cytotoxicity.
Test Material:	SUGI® macro swabs (34 mm, non-compressed), SUGI® sponge strips (35 x 7.5 x 1.5 mm, compressed)
Extract Production:	Sterile test items were transferred into the eluent (0.2 per milliliter cell culture medium containing 10 % fetal calf serum) in consideration of absorption capacities: SUGI® macro swabs 14.8 ml/g, SUGI® sponge strips 15 ml/g. Temperature and duration: 37 °C, 24 hours.
Cell Culture:	L 929 mouse fibroblasts
Controls:	Positive: Dilution series of Dimethylsulfoxide Negative: Extraction medium
Test Procedure:	96 parallel cultures per tested dilution of the extracts (100, 30, 10, 3 %). After 4 hours incubation filled with dilution series and incubates for 72 hours at 37 °C again. Dyed with crystal violet. Calculation of cell growth inhibition from the extinction values at 570 nm
Results:	Test samples and negative controls showed no signs of reactivity (Grade 0)
Conclusion:	SUGI® does not exhibit pronounced cytotoxicity

GC/MS Fingerprint (Project 14Y102, 2014)

Method:	**ISO 10993-18** Biological evaluation of medical devices, Chemical characterization of materials.
Test Material:	SUGI® macro swabs (34 mm, non-compressed), SUGI® sponge strips (35 x 7.5 x 1.5 mm, compressed)
Sample Extraction:	Medium: water, isopropyl alcohol, n-hexane. Temperature and duration: 37 °C, 72 hours. In closed glass vials
Controls:	Positive: solution of n-tetradecane dissolved in n-hexane Negative: Pure extraction
Test Procedure:	1 µl of each extract was injected into the GC (duplicate analysis per vial), separated on the capillary column and detected by MS (mass selective detector).
Results & Conclusion:	No semi-volatile organic compounds were detected above the analysis limit

Update 2022

Cytotoxicity (Project STUGC22AA0667-2, 2022)

Method:	**ISO 10993-5** Biological evaluation of medical devices, Tests for *in vitro* Cytotoxicity.
Test Material:	SUGI® - REF 31003 - Sugi Sponge Strips, rectangular - 35 x 7,5 mm, sterile, 500 pcs
Extract Production:	The test item was extracted under agitation (100 rpm) for 24 × 2 h in DMEM 10 % FBS at 37 × 1 °C, 5.0 % CO2 /95 % air in an incubator. The absorption capacity of the test item was determined (15.58 mL extraction medium/g test item) and considered for the extraction. The final weight/volume ratio in the assay was 0.1 g/mL above the absorption capacity which corresponds to 100 % extract concentration.
Cell Culture:	L929 cells (ATCC® CCL-1™, NCTC clone 929 (connective tissue mouse), clone of strain L (DSMZ))
Controls:	Positive: Latex Examination Gloves were extracted at a surface/volume ratio of 6 cm^2/mL in DMEM 10 % FBS for 24 × 2 h at 37 ± 1 °C. Negative: Polypropylene material was extracted at a surface/volume ratio of 3 cm^2/mL in DMEM 10 % FBS for 24 × 2 h at 37 ± 1 °C.
Test Procedure:	The extract of the test item was diluted three times with DMEM 10% FBS at a ratio of 2:3 in a separate 96 well plate. Then the cell culture medium of the previously seeded cells was removed and 100 µL of the dilutions of the test extract and 100 µL of the controls (100 % extract concentration), respectively, were added in quadruplicates. The tissue plate was incubated for 24 - 26 h in humidified air (5.0 % CO2 / 95 % air) at 37 ± 1 °C.
Results:	Test samples and negative controls showed no signs of reactivity (Grade 0)
Conclusion:	The cytotoxic effects of Sugi® - REF 31003 - Sugi Sponge Strips, rectangular – 35 x 7,5 mm, sterile, 500 pcs were analysed. Hereby, the test item was extracted under agitation for 24 × 2 h with cell culture medium and L929 cells were incubated for 24 - 26 h with the extract. The decrease in the activity of mitochondrial dehydrogenases of the individual cultures was then analysed as a measure for cytotoxicity and compared to those of the controls. In this study under the given conditions no leachable substances were released in cytotoxic concentrations from the test item.

GC/MS Fingerprint (Project STUGC22AA0667-3, 2022)

Method:	**ISO 10993-18** Biological evaluation of medical devices, Chemical characterization of materials.
Test Material:	SUGI® - REF 31003 - Sugi Sponge Strips, rectangular - 35 x 7,5 mm, sterile, 500 pcs
Sample Extraction:	The test item was extracted under agitation and under light protection for 72 ± 2 h at 37 ± °C with water that simulated the physiological conditions during the product-use and with two organic solvents (isopropanol and n-hexane) maximising the release of organic extractables
Controls:	Positive: Phenanthrene dissolved in isopropanol was used as reporting limit standard Negative: Extraction medium without test material
Test Procedure:	Each extract and the control standards were analysed by GC-MS. At the beginning of the sequence batch five system suitability standards were injected followed by the reporting limit standard (single injection). Afterwards, single injections of the extraction aliquots were performed. Furthermore, the system suitability standard was injected after at least every

	ten sample injections throughout the sample set, with a final injection after the last corresponding sample injection to confirm the validity of the run.
Results & Conclusion:	In this study under the given conditions no extractable substances were detected above or equal to the calculated AET value in the water extract. In this study under the given conditions no extractable substances were detected above or equal to the calculated AET value in the isopropanol extract. In this study under the given conditions no extractable substances were detected above or equal to the calculated AET value in the n-hexane extract.

Update 2023

Pyrogen test (Project STUGC23AA1814-1, 2023)

Method:	**EP 10.0 §2.6.8 Pyrogens / ISO 10993-11:2017** Biological evaluation of medical devices Part 11: Tests for systemic toxicity.
Test material:	REF 31301 - Sugi Sponge Strips, rhomboidal, 70 x 4 mm, 10 x 10 pcs., sterile
Extract production:	According to ISO 10993-12, one extract of the test sample in polar vehicle, Sodium Chloride Injection (0.9%), was prepared by submerging the device in the solvent, after complete imbibition, in order to reach a weight/volume ratio of 0.1 g/ml (the test sample was weighed before the start of the test) and incubating at (50 ± 2) °C for (72 ± 2) h in dynamic conditions (orbital stirrer).
Test population:	Specie: White rabbits Strain: New Zealand No.: 3 Sex: Male Weight: 2640 - 2730 g at the beginning of the test
Controls:	Sodium Chloride Injection, 10 ml/kg body weight (previously warmed to 37°C ± 2°C)
Test procedure:	**Dose administration (initial step)** Rabbits were fasted from the night before the pyrogen test. Rabbits were weighed and then allocated to single restraining boxes. Temperature probes were gently inserted in the rectum at least 7.5 cm. The probes were fixed via a cord at the base of the rabbit tail. The temperature of each rabbit was recorded starting from at least 60 min after placing them in restraining boxes. Animals with a temperature lower than 38°C and higher than 39.8°C were not used for the pyrogen test. The temperature was recorded for 105 min at intervals of 5 min. Rabbits showing a rise in temperature of 0.2°C in two consecutive readings during 105 min were withdrawn from the test. The sodium chloride extract was warmed for 20 min at 37°C ± 2°C before injection. Sodium Chloride Injection extract (10 ml/kg body weight at 37 ± 2°C) was injected into the ear vein of three rabbits (initial step), completing each injection within 4 min after the start of administration. The difference between temperatures in the three rabbits of the test group must be lower than 1°C. Single-use non pyrogenic disposable syringes and hypodermic needles were used to administer the test item extract.

	Post-dose procedure (temperature detection) Rectal temperature was recorded for 180 min at intervals of 5 min. The response of each rabbit (thermal raise) is calculated as the difference between the maximum temperature and initial temperature. The initial temperature of each rabbit is the mean of two temperature readings before extract injection at 75 min and at 105 min. The maximum temperature of each rabbit is the highest temperature evaluated at interval of 15 minutes in the 180 min after the injection.
Results:	The summed response of the first group of three rabbits is + 0.60 °C.
Conclusion:	On the basis of the results, the test item "REF 31301 - Sugi Sponge Strips, rhomboidal, 70 x 4 mm, 10 x 10 pcs., sterile", interpreted according to EP-current edition, MEETS THE REQUIREMENT for the absence of pyrogens.