TEST / INSPECTION REPORT

EUROLAB LABORATORY SERVICES

TÜRCERT TEKNİK KONTROL VE BELGELENDİRME A.Ş.

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Sample ID: **Gloves / Eldiven**

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Lot 3: 100170

	TEST	METHOD	Specimen	RESULT
*	**IRRITATION TEST**	**ISO 10993 - 10**	**Gloves**	**PASS**
*	**SENSITIZATION TEST**	**ISO 10993 - 10**	**Gloves**	**PASS**

Seal

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EUROLAB ® (TÜRCERT TEKNİK KONTROL VE BELGELENDİRME A.Ş.)

Environment

The requirements and standards apply to equipment intended for use in

X	Residential (domestic) environment
X	Commercial and light-industrial environment
X	Industrial environment
X	Medical environment

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ISO 10993 – Part : 10 - Skin Sensitization

Scope

This part of ISO 10993 describes the procedure for the assessment of medical devices and their constituent materials with regard to their potential to produce irritation and skin sensitization.

General principles — Step-wise approach

The available methods for testing irritation and sensitization were developed specifically to detect skin and mucous membrane irritation and skin sensitization potential. Other types of adverse effect are generally not predicted by these tests. For medical devices that are used as implants or external communicating devices, intradermal testing is more relevant in approaching the application and so for detection of irritation activity, intracutaneous testing shall be used.

Pretest considerations

Types of material

Initial considerations

It shall be taken into consideration that, during manufacture and assembly of medical devices, additional chemical components may be used as processing aids, e.g. lubricants or mould-release agents. In addition to the chemical components of the starting material and manufacturing process aids, adhesive/solvent residues from assembly and also sterilant residues or reaction products resulting from the sterilization process may be present in a finished product. Whether these components pose a health hazard/risk depends on the leakage or degradation characteristics of the finished products. These components shall be taken into account for their potential irritation/sensitization activity.

Information on chemical composition

General

Full qualitative data on the chemical constituents of the material shall be established. Where relevant to biological safety, quantitative data shall also be obtained. If quantitative data are not obtained, the rationale shall be documented and justified.

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Existing data sources

Qualitative and quantitative information on the composition shall be obtained where possible from the supplier of the starting material.

For polymers this often requires access to proprietary information; provision should be made for the transfer and use of such confidential information.

Qualitative information about any additional processing additives (for example, mould-release agents) shall also be obtained from appropriate members of the manufacturing chain, including converters and component manufacturers.

Skin sensitization tests

The test sample shall be a liquid, suspension, gel or paste such that it can be applied to the ears of the mice.

Where possible, a series of doses (dilutions) shall be investigated. Otherwise, the highest concentration prepared as a chemical solution or suspension or as an extract should be used. Systemic toxicity and excessive local skin irritation can invalidate the test results; these reactions should therefore be avoided. In certain circumstances, pre-testing can be necessary.

A commonly used vehicle for substances/chemicals is an acetone olive oil (AOO) 4:1 mixture. Liquid samples that are hydrophilic and/or do not adequately adhere to the skin of the ear should be modified to adhere to the test site. This can be obtained by adding an agent like carboxy methyl cellulose or hydroxyethyl cellulose (0,5 % w/v). For water soluble chemicals, dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF) are preferred above the surfactant Pluronic® L92. See Reference [89]. Alternatively, other extract vehicles can be used, as mentioned. See Reference [88].

The effect of additions to the extract media and/or changes in vehicle composition shall be validated and documented. This might be done by experiments using weak to moderate sensitizers as commonly used as positive control. In addition, spiking of the test sample with a positive control might be performed in order to demonstrate that the prepared extract is still able to detect the presence of potential sensitizers. The methods of extraction are described in ISO 10993-12.

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Test procedure

For chemicals, the LLNA is generally performed in a dose-response manner. For medical devices, samples to be tested may be extracts. In these cases, only a single dose is available for testing. In general, the extract can be investigated undiluted. However, when the extract contains highly toxic components, this can result in a negative response in the LLNA due to toxicity. It is therefore recommended, when investigating highly toxic extracts (see ISO 10993-5) to perform the LLNA in a dose-response manner and to dilute the extract. In order to ensure reproducibility and sensitivity of the test procedure, tests with well-known weak to moderate contact allergens, e.g. mercaptobenzothiazole, hexyl cinnamic aldehyde and benzocaine, shall be included in each assay. The examples mentioned might not be suitable for each vehicle used for sample preparation (i.e. water based vehicle); in such cases, another positive control might be selected. This shall be justified and documented. When the assay is performed frequently, positive controls do not have to be included in each assay, however they shall be included at least once every six months. The individual body weights shall be recorded at initiation and at the end of the study. In order to detect potential toxicity of the test sample, clinical observation shall be performed and recorded during the study.

Choice of test sample concentrations

Current guidelines for testing the sensitizing potential of single chemicals recommend using only one concentration for the test.

Induction

Sensitization rate is highly dependent on induction dose, which in guinea pig assays shall be mildly to moderately irritating, where possible. If the irritation threshold is not reached, then the highest possible concentration shall be used. However, it shall not interfere with the health of the animals. The induction döşe in the guinea pig assays is normally selected based on preliminary tests as described for the individual guinea pig tests. Undiluted extracts with the usual solvents for parenteral dosing need not be subjected to a preliminary test.

Important factors affecting the outcome of the test

The biochemical and physical characteristics of the test sample can influence the choice of test, since the maximization test requires intradermal injections. If the test sample cannot be injected intradermally, an alternative method shall be used. The extract solutions shall be prepared under aseptic conditions. Non-sterile solutions should not be used for intradermal applications.

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The bioavailability of the test material is influenced by the choice of vehicle. Although there is no vehicle that is optimal for all materials, a vehicle that optimizes exposure by solubilization and penetration should be selected. The concentration of test material should be the highest possible without affecting the interpretation of results. Most investigators prefer the test sample as a solution because dispersions are prone to form a sediment, making exact dosing difficult. Examples of vehicles for intradermal injection include saline, propyleneglycol and vegetable oils. Variation among results from different laboratories can have several sources. The following factors in the test procedure are important:

– ambient test conditions;
– test site on the animal;
– method of hair removal (clipping/shaving or chemical depilation);
– type of patch design;
– quantity of test material;
– quality of occlusion;
– exposure time and reading of the animals.

Animal responsiveness also varies according to genetic factors and husbandry.

Control Article

Negative Control	0.9% Sodium Chloride Injection (SC) Size:500 ml Storage Condition: Room temperature
Positive Control	2.4-Dinitrochlorobenzene (DNCB) Size: 100 g Storage Condition: Room Temperature

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Guinea pig maximization test (GPMT)

Principle

An assessment is made of the potential of the material under test to produce skin sensitization in the guinea pig using the technique applied for single chemicals in the guinea pig maximization test.

Animals and husbandry

Healthy young adult albino guinea pigs of either sex from a single outbred strain, weighing 300 g to 500 g at the start of the test, shall be used. If female animals are used, they shall be nulliparous and not pregnant.

The animals shall be acclimatized and cared for as specified in ISO 10993-2. Preliminary tests, when necessary, should be carried out on one set of animals to determine the optimal test concentrations. If the test material is powder or liquid, a minimum of ten animals shall be treated with the test sample and a minimum of five animals shall act as a control group. If a preliminary test is needed, it shall be carried out on additional animals.

For testing extracts, a minimum of ten animals shall be treated with the test sample, and a minimum of five animals shall act as a solvent control group. If a preliminary test is needed, it shall be carried out on additional animals.

If testing on ten test and five control animals is completely negative, it is unlikely that testing of a further ten plus five animals will give positive results. However, if any equivocal responses develop, rechallenge shall be carried out. If equivocal responses remain, conduct a new study on a minimum of 20 test and ten control animals.

Animal Care and Maintenance

Animal room temperature	18 – 26 °C
Animal room relative humidity	30% - 70%

Test procedure

Preparation

Clip and shave the fur on all treatment sites prior to all steps in the test procedure.

For intradermal injections, inject 0,1 ml per site.

For topical application, saturate an appropriate filter paper or absorbent gauze patch (4 cm2 to 8 cm2) with the test sample and apply the patch to the clipped skin under an occlusive dressing secured by a wrap around the torso of the animal.

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Preliminary tests

The preliminary tests are intended to determine the concentration of the test sample to be used.

Undiluted extracts using the usual solvents need not be subjected to preliminary testing.

Topically apply a range of dilutions of the test sample to the flanks of at least three animals. Remove the occlusive dressings and patches after 24 h, and assess the application sites for erythema and oedema using the Magnusson and Kligman grading scale given in Table 3. For the topical induction phase in the main test, select the highest concentration that causes mild to moderate erythema but does not otherwise adversely affect the animal. It should be recognised that for extracts of medical devices, the irritating threshold may not be obtained. In such cases, the highest concentration possible shall be used, e.g. the undiluted extract. For final products/medical devices, it may be sufficient to test only the undiluted extract. For the challenge phase in the main test, select the highest concentration that produces no erythema.

Extraction

Under aseptic conditions , samples were taken according to the sampling method Whole sampling , add additional volume of extraction vehicle that the test sample absorbs when performing the extraction .) , using the surface area data of the test article provided by the sponsor , 340 cm^2) . The extraction was performed with agitation in closed inert containers according to the extraction ratio of 6 cm : 1 mL (sample : extraction vehicle) . The extraction vehicle was 0.9 % Sodium Chloride Injection (SC) .

Test period	Actual sampling	Extract procedure			Final extract
		Extract ratio	SC	Condition	
Intraermal induction phase I	340 cm^2	6 cm^2 / 1 mL	62.1 ml	50 °C , 72 h	Not Clear
Topical Induction Phase II	340 cm^2	6 cm^2 / 1 mL	62.1 ml	50 °C , 72 h	Not Clear
Challenge Phase	340 cm^2	6 cm^2 / 1 mL	62.1 ml	50 °C , 72 h	Not Clear

The extract was stored 4°C and tested within 24 h.

Extraction

Extraction Under aseptic conditions , samples were taken according to the sampling method (Whole sampling , add additional volume of extraction vehicle that the test sample absorbs when performing the extraction .) , using the surface area data of the test article provided by the sponsor , 340 cm) . The extraction was performed with agitation in closed inert containers according to the extraction ratio of 6 cm : 1 mL (sample : extraction vehicle) . The extraction vehicle was SO .

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Test period	Actual sampling	Extract procedure			Final extract
		Extract ratio	SO	Condition	
Intraermal induction phase I	340 cm^2	6 cm^2 / 1 mL	79.9 ml	50 °C , 72 h	Clear
Topical Induction Phase II	340 cm^2	6 cm^2 / 1 mL	79.9 ml	50 °C , 72 h	Clear
Challenge Phase	340 cm^2	6 cm^2 / 1 mL	79.9 ml	50 °C , 72 h	Clear

The extract was stored 4°C and tested within 24 h.

Experimental Procedure

Animal Preparation and Grouping

Preparation Grouping On the first day of treatment, 15 guinea pigs were weighed and identified. The fur from the dorsoscapular area of the animals was removed with an electric clipper. Grouping as follow:

Group name	Group size	Gender
Test	10 animals	No particular gender is prescribed.
Negative control	5 animals	No particular gender is prescribed.

Intradermal Induction Phase I

Intradermal Induction Phase I A pair of 0.1 mL intradermal injections was made for each of the following, into each animal, at the injection sites (A, B and C) as shown in Figure 1 in the clipped intrascapular region.

Site A: a 50:50 (V/V) stable cmulsion of Freund's complete adjuvant mixed with the chosen solvent.

Site B: the test sample (undiluted extract); the control animals were injected with the solvent alone.

Site C: the test sample at the concentration used at site B, emulsified in a 50:50 (VIV) stable emulsion of Freund's complete adjuvant and the solvent (50%); the control animals were injected with an emulsion of the blank liquid with adjuvant.

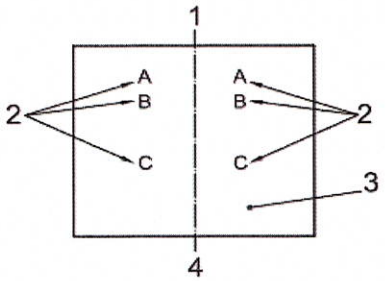

Figure 1 — Location of intradermal injection sites

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Key
1 cranial end
2 0,1 ml intradermal injections
3 clipped intrascapular region
4 caudal end

Topical Induction Phase II

The maximum concentration that can be achieved in Intradermal induction phase I did not produce irritation. Animals are pretreated with 10% sodium dodecyl sulfate (Solvent: Distilled water, Date prepared: 2019-09-30) (24±2)h before the topical induction application. At 7 d after completion of the intradermal induction phase, administer 0.5 mL test article extract by topical application to the intrascapular region of each animal, using a patch of area approximately 8 cm^2 (absorbent gauze), so as to cover the intradermal injection sites. Secure the patches with an occlusive dressing. Remove the dressings and patches after (48 ± 2) h. Treat the control animals similarly, using the blank liquid alone.

Challenge Phase

At 14±1 d after completion of the topical induction phase, challenge all test and control animals with the test sample. Absorbent gauzes (2.5 cm × 2.5 cm) were soaked respectively with 0.5 mL test article and 0.5 mL control article. Apply the test article extract and control article topically to two sites that were not treated during the induction stage. Secure with an oeclusive dressing. Remove the dressings and patches after (24 ± 2) h.

Observation of animals

Observe the appearance of the challenge skin sites of the test and control animals (24 ±2) h and (48 ± 2) hafter removal of the dressings. Full-spectrum lighting was used to wisualize the skin reactions. Describe and grade the skin reactions for erythema and oedema according to the Magnusson and Kligman grading given in the following table for each challenge site and at cach time interval.

Patch test reaction	Grading scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and/or swelling	3

— Magnusson and Kligman scale –

Evaluation of results

Magnusson and Kligman grades of 1 or greater in the test group generally indicate sensitization, provided grades of less than I are seen in control animals. If grades of I or greater are noted in control animals, then the reactions of test animals which exceed the most severe reaction in control animals presumed to be due to sensitization. If the response is equivocal, rechallenge is recommended to confirm the results from the first challenge. The outcome of the test is presented as the frequency of positive challenge results in test and control animals.

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Results

The results of skin reaction after challenge were listed in Table 3. No skin sensitization reaction was found in the skin of guinea pigs using extracts of the test article, and the positive rate of sensitization was 0%. The positive rate of sensitization in the positive control group was 100%, listed in Table 1. Clinical observations and weight changes of guinca pigs were listed in Table 4.

Conclusion;

Under the conditions of this study, the test article Gloves extract showed no significant evidence of causing skin sensitization in the guinea pig.

Group	Animal number	(24±2)h Before phase II Patch application		(24±2)h Following challenge phase		(48±2)h Following challenge phase		Positive rate after challenge phase
		left	right	Test sides	Control sides	Test sides	Control sides	
Positive Control	1	3	3	2	0	2	0	% 100
	2	3	3	2	0	2	0	
	3	3	3	2	0	3	0	
	4	3	2	2	0	2	0	
	5	3	3	2	0	2	0	
Negative Control	6	0	0	0	0	0	0	
	7	0	0	0	0	0	0	
	8	0	0	0	0	0	0	
	9	0	0	0	0	0	0	
	10	0	0	0	0	0	0	

Table 1. Guinea pig sensitization dermal reactions of positive control

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Group	Animal number	Weight (g)		Clinizal observation except dermal reactions
		Before injection	After experiment	
Positive Control	1	309	361	Normal
	2	307	374	Normal
	3	314	379	Normal
	4	352	441	Normal
	5	345	438	Normal
Negative Control	6	321	406	Normal
	7	330	412	Normal
	8	328	437	Normal
	9	326	402	Normal
	10	302	375	Normal

Table 2. Weight change and clinical observation of positive control

Group	Animal number	(24±2)h Before phase II Patch application		(24±2)h Following challenge phase		(48±2)h Following challenge phase		Positive rate after challenge phase
		left	right	Test sides	Control sides	Test sides	Control sides	
Test	1	0	0	0	0	0	0	% 0
	2	0	0	0	0	0	0	
	3	0	0	0	0	0	0	
	4	0	0	0	0	0	0	
	5	0	0	0	0	0	0	
	6	0	0	0	0	0	0	
	7	0	0	0	0	0	0	
	8	0	0	0	0	0	0	
	9	0	0	0	0	0	0	
	10	0	0	0	0	0	0	
Negative Control	11	0	0	0	0	0	0	
	12	0	0	0	0	0	0	
	13	0	0	0	0	0	0	
	14	0	0	0	0	0	0	
	15	0	0	0	0	0	0	

Table 3. Guniea pig sensitization dermal reactions

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Group	Animal number	Weight (g)		Clinizal observation except dermal reactions
		Before injection	After experiment	
Test	1	356	445	Normal
	2	349	451	Normal
	3	331	416	Normal
	4	345	427	Normal
	5	310	374	Normal
	6	321	392	Normal
	7	315	392	Normal
	8	358	447	Normal
Negative Control	9	328	412	Normal
	10	325	401	Normal
	11	317	380	Normal
	12	349	432	Normal
	13	318	383	Normal
	14	359	452	Normal
	15	321	408	Normal

Table 4. Weight change and clinical observation

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ISO 10993 - Part 10: Tests for irritation and skin sensitization

Scope

This part of ISO 10993 describes the procedure for the assessment of medical devices and their constituent materials with regard to their potential to produce irritation and skin sensitization. This part of ISO 10993 includes:

a) pretest considerations for irritation, including in silico and in vitro methods for dermal exposure;
b) details of in vivo (irritation and sensitization) test procedures;
c) key factors for the interpretation of the results.

General principles — Step-wise approach

The available methods for testing irritation and sensitization were developed specifically to detect skin and mucous membrane irritation and skin sensitization potential. Other types of adverse effect are generally not predicted by these tests. For medical devices that are used as implants or external communicating devices, intradermal testing is more relevant in approaching the application and so for detection of irritation activity, intracutaneous testing shall be used.

Pretest considerations

Types of material

Initial considerations

It shall be taken into consideration that, during manufacture and assembly of medical devices, additional chemical components may be used as processing aids, e.g. lubricants or mould-release agents. In addition to the chemical components of the starting material and manufacturing process aids, adhesive/solvent residues from assembly and also sterilant residues or reaction products resulting from the sterilization process may be present in a finished product. Whether these components pose a health hazard/risk depends on the leakage or degradation characteristics of the finished products. These components shall be taken into account for their potential irritation/sensitization activity.

Information on chemical composition

General

Full qualitative data on the chemical constituents of the material shall be established. Where relevant to biological safety, quantitative data shall also be obtained. If quantitative data are not obtained, the rationale shall be documented and justified.

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Existing data sources

Qualitative and quantitative information on the composition shall be obtained where possible from the supplier of the starting material.

For polymers this often requires access to proprietary information; provision should be made for the transfer and use of such confidential information.

Qualitative information about any additional processing additives (for example, mould-release agents) shall also be obtained from appropriate members of the manufacturing chain, including converters and component manufacturers.

Irritation tests

In vitro irritation tests

In vitro methods, the rat skin Transcutaneous Electrical Resistance (TER) test and the Human skin model test, have been internationally validated and accepted as alternative tests to assess the skin corrosivity of chemicals. National and international organizations continue working to develop and validate *in vitro* tests for skin irritancy in parallel with the search for alternative methods; others have been developing methods to quantify the responses of animals and humans in order to better define endpoints using non-invasive techniques (see F.1).

In vivo irritation tests — Factors to be considered in design and selection of *in vivo* tests

Irritation testing of medical devices can be performed with the finished product and/or extracts thereof.

Factors affecting the results of irritation studies include the following:

a) the nature of the device used in a patch test;

b) the dose of the test material;

c) the method of application of the test material;

d) the degree of occlusion;

e) the application site;

f) the duration and number of exposures;

g) the techniques used in evaluating the test.

Provisions have been included in the test procedures for evaluation of devices and materials that will have repeated and/or prolonged exposure. The study shall be designed to exaggerate the anticipated contact (time and/or concentration) in the clinical situation. This shall be borne in mind during interpretation of the result.

If the pH of the test sample is u 2,0 or W 11,5, the material shall be considered an irritant and no further testing is required. However, experimental evidence suggests that acidity and alkalinity of the test material are not the only

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factors to be considered in relation to the capacity of a material to produce severe injury. The concentration of the test material, its period of contact, and many other physical and chemical properties are also important.

In exceptional cases where further risk characterization/assessment is needed, it might be necessary to test materials which are either an irritant or have a pH outside the range mentioned above. These cases shall be justified and documented.

Animal irritation test

Principle

An assessment is made of the potential of the material under test to produce dermal irritation in a relevant animal model.

The rabbit is the preferred test animal.

Animal Care and Maintenance	
Animal room temperature	18-26 °C
Animal room relative humidity	30%-70%
Lights	12 hours light/dark cycle, full spectrum lighting
Selection	Only healty animal were selected

Test material

The sensitivity of the assay shall be demonstrated. This can be done by including a positive control in the assay. However, the use of a positive control to confirm sensitivity is only warranted when the testing laboratory has not within the previous six months produced positive results using the test method.

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Animals and husbandry

Three healthy young adult albino rabbits of either sex from a single strain, weighing not less than 2 kg, shall be used. If irritation is anticipated, consideration shall be given to testing in one animal first. Unless a welldefined positive response [score greater than 2 for either erythema or oedema is observed, a minimum of two additional animals shall be used. If the response in the test using the minimum of three animals is equivocal, further testing shall be considered.

Used Positive Control	(SDS) Sodium Dodecyl Sulfate
Used Negative Control	PBS (Phosphate Buffer Saline)

Polar	(0.9% saline solution of NaCl)
Non-Polar	(Sesame Oil of pharmaceutical grade) solvents

Negative control (NC) acceptance criteria: The NC data meet the acceptance criteria if the mean OD value of the 3 tissues is ≥ 1.2 at 570 nm according to the historical database. The Standard Deviation value is considered as valid if it is ≤ 18%.

Positive control (PC) acceptance criteria: The PC data meet the acceptance criteria if the mean viability, expressed as % of the NC, is < 40 % and the Standard Deviation value is ≤ 18 %.

Experimental Design

Pretreatment

No pretreatment requirement.

Extraction

Under aseptic conditions, samples were taken according to the sampling method (Whole sampling add additional volume of extraction vehicle that the test sample absorbs when performing the extraction, using the surface area data of the test article provided by the sponsor, 340 cm^2). The extraction was performed with agitation in closed inert containers according to the extraction ratio of 6 cm^2: 1 mL (sample: extraction vehicle). The extraction vehicle was SC.

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Test Period	Actual Sampling	Extract Procedure			Final Extract
		Extract Ratio	Extraction Volume	Condition	
Polar test extract	Surface area 340 cm^2	6cm^2 / 1 ml	62.1 ml	50 °C , 72h	Clear
Polar negative control	/	/	10.0 ml	50 °C , 72h	Clear

Test Period	Actual Sampling	Extract Procedure			Final Extract
		Extract Ratio	Extraction Volume	Condition	
Non - Polar test extract	Surface area 340 cm^2	6cm^2 / 1 ml	62.1 ml	50 °C , 72h	Clear
Non - Polar negative control	/	/	10.0 ml	50 °C , 72h	Clear

Test period	Actual Sampling	Extract Procedure			Final Extract
		Extract Ratio	Volume of Extraction Vehicle	Condition	
Test	340 cm^2	6 cm^2:1 ml	62.1 ml	37 °C, 72 h	Clear
Negative Control	30 cm^2	3 cm^2:1 ml	10.0 ml	37 °C, 72 h	Clear
Blank Control	-		10.0 ml	37 °C, 72 h	Clear
Positive Control	0.5 g	1.0 g:100 ml	50.0 ml	37 °C, 72 h	Not clear

Reaction	Irritation Score
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet-redness) to eschar formation preventing grading of erythema	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Well-defined oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond exposure area)	4
Maximal possible score for irritation	8
Other adverse changes at the skin sites shall be recorded and reported.	

Table 1 — Scoring system for skin reaction

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Preparation of animals

The condition of the skin is a critical factor. Use only animals with healthy intact skin. Fur is generally clipped within 24 h to 4 h of testing on the backs of the animals, a sufficient distance on both sides of the spine for application and observation of all test sites (approximately 10 cm × 15 cm). Fur may be re-clipped to facilitate observation and/or to accommodate repeated exposures.

Application of test sample

Application of powder or liquid sample

Apply 0,5 g or 0,5 ml of the test material directly to each test skin site as shown in Figure 1. For solid and hydrophobic materials, there is no need for moistening.

Cover the application sites with a 2,5 cm × 2,5 cm non-occlusive dressing (such as an absorbent gauze patch) and then wrap the application site with a bandage (semi-occlusive or occlusive) for a minimum of 4 h. At the end of the contact time, remove the dressings and mark the positions of the sites with permanent ink. Remove residual test material by appropriate means, such as washing with lukewarm water or other suitable nonirritating solvent, and careful drying.

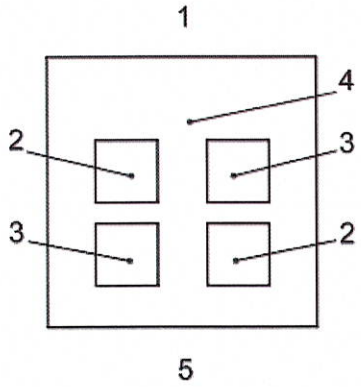

Figure 1 — Location of skin application sites

Key
1 cranial end
2 test site
3 control site
4 clipped dorsal region
5 caudal end

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Application of solid sample

Apply the samples of the test material directly to the skin on each side of each rabbit as shown in Figure 1. Similarly, apply the control samples to each rabbit. When testing the test material shall be moistened sufficiently with water or, where necessary, an alternative solvent, to ensure good contact with the skin (see Annex A). When solvents are used, the influence of the solvent on irritation of skin caused by the test material shall be taken into account. Cover the application sites with 2,5 cm × 2,5 cm non-occlusive dressings (such as a gauze patch) and then wrap the application sites with a bandage (semi-occlusive or occlusive) for a minimum of 4 h. At the end of the contact time, remove the dressings and mark the positions of the sites with permanent ink. Remove residual test material by appropriate means, such as washing with lukewarm water or other suitable non-irritating solvent and careful drying.

Observation of animals

General

Use of natural or full-spectrum lighting is highly recommended to visualize the skin reactions. Describe and score the skin reactions for erythema and oedema according to the scoring system given in Table 1, for each application site at each time interval, and record the results for the test report.

Single-exposure test

For single-exposure tests, record the appearance of each application site at (1 ± 0,1) h, (24 ± 2) h, (48 ± 2) h and (72 ± 2) h following removal of the patches. Extended observation can be necessary if there are persistent lesions, in order to evaluate the reversibility or irreversibility of the lesions over a period of time not exceeding 14 d.

Repeated-exposure test

Repeated exposure shall only be carried out after completion of an acute single-exposure test [after at least (72 ± 2) h of observation].

For repeated-exposure tests, record the appearances of the application site at (1 ± 0,1) h after removal of the patches and immediately prior to the next application. The number of exposures can vary. After the last exposure, note the appearance of each application site at (1 ± 0,1) h, (24 ± 2) h, (48 ± 2) h and (72 ± 2) h following removal of the patches. Extended observation can be necessary if there are persistent lesions, in order to evaluate the reversibility or irreversibility of the lesions. This need not exceed a period of 14 d.

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Evaluation of results

For single exposure tests, determine the primary irritation index (PII) as follows.

Use only (24 ± 2) h, (48 ± 2) h and (72 ± 2) h observations for calculations. Observations made prior to dosing or after 72 h to monitor recovery are not used in the determination.

After the 72 h grading, all erythema grades plus oedema grades (24 ± 2) h, (48 ± 2) h and (72 ± 2) h are totalled separately for each test sample and blank for each animal. The primary irritation score for an animal is calculated by dividing the sum of all the scores by 6 (two test/observation sites, three time points).

To obtain the primary irritation index for the test sample add all the primary irritation scores of the individual animals and divide by the number of animal.

Mean score	Response category
0 to 0,4	Negligible
0,5 to 1,9	Slight
2 to 4,9	Moderate
5 to 8	Severe

Table 2 — Primary or cumulative irritation index categories in a rabbit

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RESULT

DERMAL OBSRVATIONS (Polar extract)						
Rabbit no	Group		Interval(hours):score=left side/right side			
			1±0.1	24±2	48±2	72±2
1	Test article	Erythema	0/0	0/0	0/0	0/0
		Oedema	0/0	0/0	0/0	0/0
	Negative control	Erythema	0/0	0/0	0/0	0/0
		Oedema	0/0	0/0	0/0	0/0
2	Test article	Erythema	0/0	0/0	0/0	0/0
		Oedema	0/0	0/0	0/0	0/0
	Negative control	Erythema	0/0	0/0	0/0	0/0
		Oedema	0/0	0/0	0/0	0/0
3	Test article	Erythema	0/0	0/0	0/0	0/0
		Oedema	0/0	0/0	0/0	0/0
	Negative control	Erythema	0/0	0/0	0/0	0/0
		Oedema	0/0	0/0	0/0	0/0
Primary irritation index			0			

DERMAL OBSRVATIONS (Non-polar extract)						
Rabbit no	Group		Interval(hours):score=left side/right side			
			1±0.1	24±2	48±2	72±2
1	Test article	Erythema	0/0	0/0	0/0	0/0
		Oedema	0/0	0/0	0/0	0/0
	Negative control	Erythema	0/0	0/0	0/0	0/0
		Oedema	0/0	0/0	0/0	0/0
2	Test article	Erythema	0/0	0/0	0/0	0/0
		Oedema	0/0	0/0	0/0	0/0
	Negative control	Erythema	0/0	0/0	0/0	0/0
		Oedema	0/0	0/0	0/0	0/0
3	Test article	Erythema	0/0	0/0	0/0	0/0
		Oedema	0/0	0/0	0/0	0/0
	Negative control	Erythema	0/0	0/0	0/0	0/0
		Oedema	0/0	0/0	0/0	0/0
Primary irritation index			0			

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Rabbit no	Group		Interval(hours):score=left side/right side			
			1±0.1	24±2	48±2	72±2
1	Positive control	Erythema	0/0	1/1	2/3	3/4
		Oedema	0/0	2/1	2/3	3/3
	Negative control	Erythema	0/0	0/0	0/0	0/0
		Oedema	0/0	0/0	0/0	0/0
2	Positive control	Erythema	0/1	1/2	3/3	4/3
		Oedema	0/0	1/1	3/2	4/3
	Negative control	Erythema	0/0	0/0	0/0	0/0
		Oedema	0/0	0/0	0/0	0/0
3	Positive control	Erythema	1/0	1/2	3/2	4/3
		Oedema	0/1	2/2	3/3	3/4
	Negative control	Erythema	0/0	0/0	0/0	0/0
		Oedema	0/0	0/0	0/0	0/0
Primary irritation index			4.9			

Conclusion;
According to ISO 10993-10 standard for " Biological evaluation of medical devices — Part 10: Tests for irritation and skin sensitization " , Irritation was not observed during the test.

Result

All animals were survived and no abnormal signs were observed during the study. According what observed, the skin reaction of non-polar extract on testing side did not exceed that on the control side. Thus, the final test article score was calculated to be 0.

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IMAGE

*** END OF REPORT***

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