TEST REPORT

EN 455

Medical gloves for single use

Report Number.....:	**WUX202003170513S**
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Compiled by (+signature).....:	Technique principal Andy Liu
Approved by (+signature).....:	Manager Tony Bi
Date of issue.....:	Mar. 20, 2020
Total number of pages.....:	18 pages
Testing laboratory .....:	Shenzhen Huacetong Testing and certification Co., Ltd.
Address .....:	Building B, Xinbaosheng, No.233, Xixiang Street, Bao'an District, Shenzhen, China
Testing location .....:	As above
Applicant's name.....:	
Address.....:	
Test specification:	
Standard.....:	EN ISO 14971: 2012 EN ISO 15223-1: 2012 EN 455-1:2000 EN 455-2: 2015 EN 455-3: 2006
Test procedure.....:	N/A
Non-standard test method……..:	N/A
Test Report Form No.....:	EN 455
Test Report Form(s) Originator.....:	N/A
Master TRF.....:	N/A

Test item description	:	Disposable examination gloves
Trade Mark	:	Dihang
Manufacturer	**:**	
Address	:	
Model/Type reference	:	VINYL-011/020, NITRILE-021/030, LATEX -031/040
Ratings	:	--

Clause(s) Test(s) Test Remarks Result

Summary of testing:	
Tests performed (name of test and test clause): - BS EN ISO 20345:2011 The submitted samples were found to comply with the requirements of above specification.	Testing location: Building B, Xinbaosheng, No.233, Xixiang Street, Bao'an District, Shenzhen, China

Summary of testing: **Tests performed (name of test and test clause):**				**Testing location:**
5	Water tightness test for detection of holes	Applicable	Pass	1)
5.1	Referee testing	Applicable	Pass	1)
5.2	Routine testing	Applicable	Pass	1)
5.2	Force at break	Applicable	Pass	1)
5.1	Endotoxins	Applicable	Pass	1)
5.2	Powder	Applicable	Pass	1)

Test item particulars.....:	
Temperature.....:	23°C±2°C
Relative humidity.....:	50％R. H.
Atmospheric pressure.....:	(9.0±0.2)kPa
Mass of the equipment (kg).....:	<500g
Possible test case verdicts:	
- test case does not apply to the test object.....:	N/A
- test object does meet the requirement.....:	P (Pass)
- test object does not meet the requirement.....:	F (Fail)
Testing.....:	
Date of receipt of test item.....:	Mar. 17, 2020
Date (s) of performance of tests.....:	Mar. 17, 2020 –Mar. 20, 2020

Clause(s)	Test(s)	Test Remarks	Result

General remarks:

The test results presented in this report relate only to the object tested.
This report shall not be reproduced, except in full, without the written approval of the Issuing testing laboratory.
"(See Enclosure #)" refers to additional information appended to the report.
"(See appended table)" refers to a table appended to the report.

Throughout this report a ☒ comma / ☐ point is used as the decimal separator.

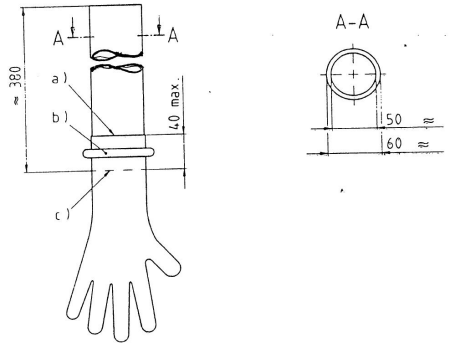
Clause numbers between brackets refer to clauses in EN 455-1, EN 455-2, EN 455-3

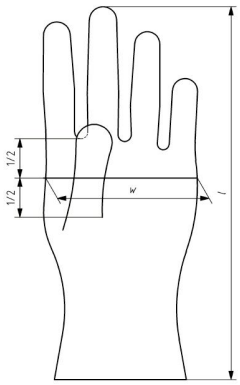
Attachment No. 1: 1 page of photo.

General product information:

Disposable examination gloves for single use.

Clause(s) Test(s) Test Remarks Result

Clause(s)	Test(s)	Test Remarks	Result
EN 455-1	Requirements and testing for freedom from holes		
5	Water tightness test for detection of holes		P
5.1	Referee testing		P
	Vertically position a filling tube of dimensions shown in Figure 1 or of dimensions to fit the glove and such that the tube is capable of holding any of the 1000 ml of water that may exceed the natural fill volume of the glove		P
	Attach the glove to the filling tube, overlapping the cuff by a maximum of 40mm over the end of the tube and secure it by suitable means to obtain a watertight seal without damaging the glove		P
	Add 1000ml ±50ml of water at a temperature of (15 to 35) °C into the open end of the filling tube, allowing the water to pass freely into the glove	25°C 1000ml of water	P
	Some of the water may remain in the filling tube depending on the glove being tested		N/A
	Immediately inspect the glove visually for water leakage. Allow the glove to hang and visually inspect the glove for water leakage again after a period of 2min to 3min	After 3min	P
	if, because of distension of the glove, the water does not rise to within 40 mm of the cuff end, raise the glove after the second inspection by a suitable means until the water level reaches 40 mm from the cuff end. Inspect visually the previously untested portion of the glove after a further period of 2min to 3min	After 3min	P
	Disregard leakages within 40 mm of the cuff		P
5.2	Routine testing		N/A
	Routine testing shall be either by the water tightness test given in 5.1 or by another test which is validated against this test		N/A
	Figure 1 		N/A

Clause(s)	Test(s)	Test Remarks	Result
	Visually inspect	No any water drops on the surface of the glove.	P
EN 455-2	Requirements and testing for physical properties		P
3	Terms and definitions		P
	medical gloves for single use		P
	surgical gloves		N/A
	examination gloves, procedure gloves		N/A
4	Dimensions		P
4.1	General		P
	measured as described in 4.2 and 4.3 taking 13 samples from each lot		P
			N/A
4.2	Length		P
	Measure the length dimension *l*		P
4.3	Width		P
	Measure the width dimension *w*		P
	Dimensions of surgical gloves **Size** / **Median length[a]** *l* in mm / **Median width[b c]** *w* in mm 5 / ≥ 250 / 67 ± 4 5,5 / ≥ 250 / 72 ± 4 6 / ≥ 260 / 77 ± 5 6,5 / ≥ 260 / 83 ± 5 7 / ≥ 270 / 89 ± 5 7,5 / ≥ 270 / 95 ± 5 8 / ≥ 270 / 102 ± 6 8,5 / ≥ 280 / 108 ± 6 9 / ≥ 280 / 114 ± 6 9,5 / ≥ 280 / 121 ± 6	Dimensions of examination, procedure gloves	N/A
	Size / **Median length[a]** *l* in mm / **Median width[b c]** *w* in mm Extra Small / ≥ 240 / ≤ 80 Small / / 80 ± 10 Medium / / 95 ± 10 Large / / 110 ± 10 Extra Large / / ≥ 110	Dimensions of examination, procedure gloves	P
	Measure *l*	≥240mm	P
	Measure *w*	95±10mm (Medium)	P

Clause(s)	Test(s)	Test Remarks	Result
5	Strength		P
5.1	General		P
	the strength of the glove is tested as described in 5.2 at a temperature of (23 ± 2) °C and a relative humidity of (50 ± 5) % R.H.		P
5.2	Force at break		P
5.2.1	Ageing and shelf life requirements are described in EN 455-4:2009.		N/A
5.2.2	Obtain one dumb-bell test piece from each of 13 gloves taken from a single lot (from seven pairs of gloves where applicable) using a cutter as specified in Figure 2 from the palm, back of the hand or cuff areas of each glove in the test sample, avoiding textured areas if possible and taking the test pieces in the direction of the longitudinal axis of the glove.		P
5.2.3	Determine the force at break of the 13 test pieces after conditioning for a minimum of 16 h. The tensometer should be equipped with a load cell appropriate for the strength of the sample under test, with jaws that firmly grip but do not damage the test specimen and with a crosshead speed of 500 mm/min.		P
	If a test piece breaks at the shoulder, it is not necessary to repeat the test on another test piece.		P
5.2.4	a) Determine the single wall thickness (tf) of the same glove as in 5.2.2 at a point on the middle finger within (13 ± 3) mm of the fingertip by measuring the double wall thickness as described in method A of ISO 23529:2010, Clause 7.1, using a gauge with a foot pressure of (22 ± 5) kPa. Take the single wall thickness as one half of the measured double wall thickness.		P
	b) Measure the thickness of the dumb-bell test pieces (tx) as described in method A of ISO 23529:2010, Clause 7.1, using the gauge described in 5.2.4 a).		P
	c) Compare the values of tf and tx. If tf/tx ⩾ 0,9, no correction to the measured force at break is necessary. If tf/tx < 0,9, correct the measured value by multiplying the measured force at break (see 5.2.3) by a factor of tf/tx.		P
5.2.5	Record the force at break, in N	>10N	P
	Force at break in Newton **Surgical gloves a)** / **Examination/procedure gloves b)** / **c)** **Throughout shelf life tested according to 5.2 and within 12 months of manufacture tested according to 5.3**: ≥ 9,0 / ≥ 6,0 / ≥ 3,6 a) Requirements for all surgical gloves. b) Requirements for all examination gloves, except gloves made from thermoplastic materials (e.g. polyvinylchloride, polyethylene).. c) Requirements for gloves made from thermoplastic materials (e.g. polyvinylchloride, polyethylene).		P

Clause(s)	Test(s)	Test Remarks	Result
5.3	Force at break after challenge testing		P
5.3.1	Gloves packaged in unit packages or gloves taken from bulk packages shall be placed for a period of seven days at a temperature of 70 °C in an oven.		P
5.3.2	Measure the force at break	>10N	P
7	Labelling		P
	In addition to labelling requirements defined in other parts of EN 455 manufacturers shall label the glove and/or the packaging with the date of manufacture in accordance with **EN ISO 15223-1:2012** and EN 1041:2008+A1:2013. Date of manufacture is defined as the packaging date.		P
EN 455-3	Requirements and testing for biological evaluation		P
4	Requirements		P
4.1	Medical gloves for single use shall be evaluated as described in the EN ISO 10993 series. Part 1 of this series describes the general principles governing the biological evaluation of medical devices and shall be used to select the appropriate tests as described in other parts of the series.		N/A
	A risk management process in accordance with **EN ISO 14971** shall be established.		P
4.2	Chemicals		P
	Gloves shall not be dressed with talcum powder	No used	P
	The manufacturer shall disclose, upon request, a list of chemical ingredients either added during manufacturing or already known to be present in the product such as accelerators, antioxidants and biocides, that are known to cause adverse health effects based on current data.		P
4.3	Endotoxins		P
	The manufacturer shall monitor the endotoxin contamination of sterile gloves using the test method specified if the gloves are labelled with low endotoxin content'. For such labelled gloves the endotoxin content shall not exceed the limit of 20 endotoxin units per pair of gloves.		P
4.4	Powder		P
	Any glove containing more than 2 mg powder is a powdered glove.	<1mg	P
4.5	Proteins, leachable	Nitrile	N/A

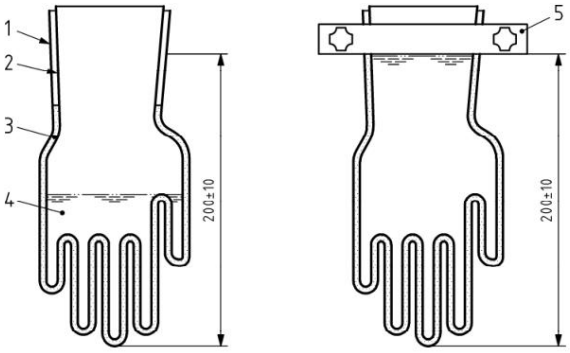
Clause(s)	Test(s)	Test Remarks	Result
	The manufacturer shall monitor the process limit of leachable protein in the finished gloves containing natural rubber latex by the method specified in 5.3 and described in Annex A. The documentation of these results shall be retained. The results of the test and applied test method shall be made available on request. The leachable protein level shall be "As Low As Reasonably Practicable" (ALARP).		N/A
4.6	Labelling		N/A
	medical gloves containing natural rubber latex shall be labelled at least on the packaging, of the smallest packaging unit with the following symbol  NITRILE VINYL		N/A
	The labelling shall include the following or equivalent warning statement together with the symbol: Product contains natural rubber latex which may cause allergic reactions, including anaphylactic responses;		N/A
	The labelling shall include a prominent indication of whether the glove is powdered or powder-free		N/A
	sterile powdered gloves shall be labelled with the following or equivalent: CAUTION: Surface powder shall be removed aseptically prior to undertaking operative procedures in order to minimize the risk of adverse tissue reactions;		N/A
	for any medical glove containing natural rubber latex the product labelling shall not include: - any term suggesting relative safety, such as low allergenicity, hypo allergenicity or low protein; -any unjustified indication of the presence of allergens;		N/A
	if the manufacturer labels the gloves with the protein content, the process limit, measured as specified in 5.3 shall be given.		N/A
5	Test methods		P
5.1	Endotoxins		P

Clause(s)	Test(s)	Test Remarks	Result
	The outside surface of a pair of gloves is extracted with 40 ml of endotoxin-free water (Water LAL, European Pharmacopoeia, for not less than 40 min and not more than 60 min at a temperature between 37 °C and 40 °C in a way to ensure that all surfaces come into contact with the extraction medium. The extract is centrifuged, if necessary, for 15 min at 2000 g to remove particles after which the liquid component is decanted and tested for endotoxin immediately afterwards.	No endotoxins	P
5.2	Powder	<1mg	P
	The test method for the determination of powder residues described in EN ISO 21171 shall be used		P
	At 25°C on two gloves randomly selected		P
	Take a 90mm, 2,7um pore size filter and place it in the desiccator for not less than 30min. remove the filter and immediately weigh it on the balance, determining the mass to the neatest 0,1mg. Record in grams (m0)		P
	Remove as much water from the filter as possible by suction. Discard the filtrate. Carefully remove the filter and transfer it to a washed and dried watch glass or petri dish. Dry in an oven at 100°C for 1h then transfer to desiccator to cool for not less than 30 min. weigh the filter immediately after removal from the desiccator to minimize re-adsorption of moisture. Record the mass, in grams, to the nearest 0,1mg(m1)		P
5.3	Proteins, leachable		N/A
	The test method for the analytical determination of leachable protein shall be the modified Lowry method given in Annex A or a suitably validated method which has been correlated against the modified Lowry method.		N/A
	The area which shall fulfil the requirements given in this subclause shall have a minimum height in accordance with Table 10, when measured from the horizontal surface beneath the sole.		N/A
Annex A	Method for the determination of aqueous extractable proteins in natural rubber gloves using the modified Lowry assay		N/A
	Spectrophotometric measurements are performed at a fixed wavelength in the range 600 nm to 750 nm.	190-195nm	N/A
A.3.2	Reagents		N/A
A.3.2.1	N-tris-[Hydroxymethyl]-methyl-2-aminoethanesulfonic acid (TES), hemi sodium salt.		N/A
A.3.2.2	Extraction buffer		N/A
	0,1 M, prepared by dissolving 24 g TES in 1 l water. Any equivalent buffering system can be used provided the solution has sufficient buffering capacity to hold a pH of 7,4 ± 0,2 in the glove extracts.		N/A

Clause(s)	Test(s)	Test Remarks	Result
A.3.2.3	Dye solution		N/A
	Bromophenol blue, sodium salt solution, prepared by dissolving 100 mg bromophenol blue in of water. Prepare a fresh solution every four weeks.		N/A
A.3.3	Lowry protein assay reagents		N/A
A.3.3.1	Reagent A		N/A
	Copper reagent (alkaline copper tartrate or copper citrate solution).		N/A
A.3.3.2	Reagent B		N/A
	Diluted Folin reagent.		N/A
A.3.4	Sodium hydroxide		N/A
	0,1 M aqueous solution.		N/A
A.3.5	Sodium deoxycholate (DOC)		N/A
	3,47 mM, prepared by dissolving 0,15 g sodium deoxycholate in water and diluting with water to 100 ml. Do not use this solution more than four weeks after it has been prepared.		N/A
A.3.6	Trichloroacetic acid (TCA),		N/A
	4,4 mM in water, prepared by dissolving 72 g TCA in water and diluting with water to 100 ml.		N/A
A.3.7	Phosphotungstic acid (PTA)		N/A
	prepared by dissolving 72 g PTA in water and diluting with water to 100 ml. Do not use this solution more than four weeks after it has been prepared.		N/A
A.3.8	Ovalbumin, from chicken egg 2) lyophilized, salt-free		N/A
A.4	Apparatus, powder-free.		N/A
A.4.1	Synthetic gloves		N/A
A.4.2	Centrifuge		N/A
	capable of reaching at least 6000 g		N/A
A.4.3	Centrifuge tubes		N/A
	30 ml or 50 ml polypropylene tubes with a low protein binding capacity of 10 µg per tube or less. Do not use glass equipment because of surface absorption of proteins.		N/A
A.4.4	Filter units		N/A
	Single use, with 0,22 µm pore size and a low protein binding capacity of 10 µg per filter or less.		N/A
A.4.5	Syringes		N/A
	Disposable, 20 ml, made of polyethylene or polypropylene.		N/A
A.4.6	Micro tubes, 2 ml, made of polypropylene.		N/A
A.4.7	Quartz cuvett, of 1 cm path length.		N/A
A.4.8	Microtitre plate		N/A

Clause(s)	Test(s)	Test Remarks	Result
	with 96 wells, flat bottomed, made of polystyrene, or disposable cuvettes		N/A
A.4.9	Disposable cuvettes		N/A
	1,5 ml semi-micro, 1 cm path length, made of polystyrene		N/A
A.4.10	Microplate reader		N/A
	Operating at a wavelength in the range 600 nm to 750 nm		N/A
A.4.11	Spectrophotometer	190-195nm	N/A
	Operating in the wavelength range 230 nm to 750 nm.		N/A
A.4.12	Vortex mixer.		N/A
A.4.13	Micropipettes, with disposable polypropylene tips.		N/A
A.4.14	Clamps		N/A
	For sealing gloves watertight during extraction. Pairs of aluminium bars lined with foam rubber and which can be screwed together (see Figure A.1) or 170 mm long plastic clips for haemodialysis are suggested.		N/A
A.4.15	Shaker		N/A
A.5	Measurement of protein binding capacity		N/A
A.5.1	General		N/A
A.5.2	Protein binding capacity of centrifuge tubes		N/A
A.5.2.4	Calculate the average absorbed ovalbumin from the expression:		N/A
	$O = \frac{10(R-T)}{5}$		N/A
A.5.3	Protein binding capacity of filter units		N/A
A.5.3.4	Calculate the average absorbed ovalbumin from the expression:		N/A
	$O = \frac{10(R-T)}{5}$		N/A
A.6	Procedure		N/A
A.6.1	General		N/A
A.6.2	Extraction procedure		N/A
A.6.2.1	Use synthetic gloves to handle the glove samples used for the extraction.		N/A
A.6.2.2	Pour into the inner glove a sufficient quantity of dye solution to fill all five fingers. Introduce 25 ml extraction buffer) at (25 ± 5) °C between inner and outer gloves.		N/A
A.6.2.3	Fix the gloves to the shaker and shake for (120 ± 5) min at (25 ± 5) °C.		N/A

Clause(s)	Test(s)	Test Remarks	Result
A.6.2.4	Remove the clamp and separate the gloves carefully. Take care not to contaminate the extract with the dye solution. If the extract is coloured blue, it shall be discarded and the extraction repeated with fresh gloves.		N/A
A.6.2.6	Cut the section of the cuff above the 20 cm mark from the extracted outer glove, wipe the liquid off the surface with tissue, allow to dry at room temperature and weigh it to the nearest 0,1 g (m 2). Calculate the mass (m) of the extracted part of the glove as follows:		N/A
	$m = m_1 - m_2$		N/A
A.6.3	Protein standard		N/A
A.6.3.1	Stock protein solution		N/A
A.6.3.2	Protein standard solutions		N/A
A.6.4	Precipitation and concentration of protein		N/A
A.6.4.1	Carry out the procedure in duplicate at (25 ± 5) °C.		N/A
A.6.4.2	Accurately transfer 1 ml each of the blank, protein standard solutions (A.6.3.2) and the four glove extracts (A.6.2.5) into micro tubes (A.4.6). Add 0,1 ml of DOC (A.3.5), mix by vortexing and allow to stand for 10 min. Add 0,1 ml of TCA (A.3.6) and 0,1 ml PTA (A.3.7), mix by vortexing and allow to stand for a further 30 min.		N/A
A6.4.3	Centrifuge at 6000 g for 15 min. Decant the supernatant liquid and drain for 5 min by inverting each centrifuge tube on an absorbent paper.		N/A
A.6.5	Colour development		N/A
A.6.5.1	The method described here is adapted to the commercial kit used for validation. Other kits or reagents prepared from off-shelf chemicals can require other volumes and incubation times.		N/A
A.6.5.2	Add 0,125 ml Reagent A into each micro tube containing the re-dissolved protein solutions including the blank.		N/A
	Mix well. Add 1 ml Reagent B, cap the tube, vortex and allow the colour to develop fully for 30 min. Should precipitation occur at this stage, centrifuge or filter to remove the precipitate before measuring the absorbance.		N/A
A.6.6	Measurement		N/A
A..6.6.1	Micro-plate reader		N/A
	Pipette a consistent volume of the solution to the well of a microtitre plate so that the well is almost full, e.g. 490 µ l in a 500 µ l well. Measure the absorbance versus the blank at a specific wavelength in the range of 600 nm to 750 nm.		N/A
A.6.6.2	Transfer the solution to a cuvette and measure the absorbance against the blank at a specific wavelength in the range 600 nm to 760 nm.		N/A

Clause(s)	Test(s)	Test Remarks	Result
A.7	Expression of results		N/A
A.7.1	Calculation		N/A
A.7.2	Results		N/A
	$P = \frac{(V \cdot C \cdot F)}{m}$		N/A
	P is extractable protein in µg/g of glove;		N/A
	V is the volume of extraction medium used in ml;		N/A
	C is the protein concentration of the extract in µg/ml;		N/A
	F is the dilution factor		N/A
	1 outer glove (glove 1)		N/A
	2 inner glove (glove 2)		N/A
	3 extraction buffer		N/A
	4 dye solution		N/A
	5 glove clamp		N/A
			N/A
A.7.3	Statistical information		N/A
			N/A

	Number of measurements	Number of extracts	Number of days	Mean in µg/ml	Repeatability coeffiecient of variation in % (within laboratories)	Reproducibility coefficient of variation in % (between laboratories)
Glove extract	8 triplicates	1 used by all participants	1	63,9	4,9	9,6
Glove extract	15 triplicates		5	61,7	6,8	6,3
Glove A	5 triplicates	5	1	88,8	7,9	22,5
Glove A	5 triplicates	5	5	84,5	6,1	20,3
Glove B	3 triplicates	3	1	109	20,2	23,3
Glove C	3 triplicates	3	1	727	8,3	23,0
Glove D	3 triplicates	3	1	46,5	10,1	31,8
Mean extract without extraction procedure					5,0	8,0
Mean whole procedure (glove A to D)					10,5	24,2

A.8	References		N/A
	Lowry OH, Rosebrough, NJ, Farr AL, Randall RJ, Protein measurement with Folin Phenol reagent. J Biol Chem 1951 : 193 : 265-275		N/A
	ASTM D 5712:1995, Standard test method for analysis of protein in natural rubber and its products		N/A
	Kidwai SA, Ansari AA, Salahuddin, Effect of succinylation (3-carboxypropionylation) on the conformation and immunological activity of ovalbumin. Biochem J 1976 : 155 : 171-180		N/A

Clause(s)	Test(s)	Test Remarks	Result
Annex B	Immunological methods for the measurement of natural rubber latex allergens		N/A
B.1	Introduction		N/A
B.2	Natural rubber latex allergens in manufactured rubber products		N/A
B.3	Methods for measuring natural rubber latex allergens		N/A
B.3.1	Qualitative methods		N/A
B.3.2	Semiquantitative methods		N/A
B.3.2.1	Skin prick testing in voluntary latex-allergic subjects		N/A
B.3.2.2	IgE-ELISA inhibition (also known as RAST-Inhibition)		N/A
B.3.3	Specific quantitative methods		N/A
B.3.3.1	Capture enzyme immunoassays (EIA) for NRL allergen quantification		N/A
B.3.3.2	Background		N/A
B.3.3.3	Description of capture EIA methods		N/A
B.3.3.4	Performance of the capture EIAs in comparison with IgE-based allergen assays		N/A
B.4	Conclusion		N/A
B.5	References		N/A
Annex C	Amino acid analysis (AAA) by high pressure liquid chromatography (HPLC)		N/A
C.1	Background		N/A
C.2	Principles of the determination of proteins by HPLC		N/A
C.3	Material		N/A
C.3.1	DL-Norvalin		N/A
C.3.2	HCl 30 % Suprapur		N/A
C.3.3	Amino acid standard		N/A
C.3.4	Methanol protein sequencing grade		N/A
C.3.5	o-Phthaldialdehyde (OPA)		N/A
C.3.6	Boric acid		N/A
C.3.7	Ethylendiaminetetratacetic acid, disodium salt (EDTA)		N/A
C.3.8	Potassium phosphate monobasic		N/A
C.3.9	Sodium phosphate dibasic		N/A
C.3.10	Sodium phosphate monobasic		N/A
C.3.11	3-mercaptopropionic acid		N/A
C.3.12	Separation column: Hypersil ODS 3 µm, 150 x 4,6 mm, pre-tested for OPA application		N/A
C.3.13	Precolumn: Hypersil ODS, 3 µm, 5 x 4,6 mm		N/A
C.3.14	Water at least Milli-Q or equivalent quality		N/A

Clause(s)	Test(s)	Test Remarks	Result
C.3.15	Filter unit 0,2 µm pore size		N/A
C.3.16	Tetrahydrofuran (THF) gradient grade for liquid chromatography		N/A
C.3.17	Acetonitril gradient grade for liquid chromatography		N/A
C.3.18	2 ml screw capped polypropylene vessels		N/A
C.3.19	Sodium carbonate		N/A
C.3.20	Sodium hydroxide or potassium hydroxide pellets		N/A
C.4	Buffers and solutions		N/A
C.4.1	Norvalin-100		N/A
	11,7 mg norvalin in 1 ml water = 100 mM norvalin		N/A
C.4.2	Norvalin-1		N/A
	100 µl norvalin-100 in 10 ml water = 1 mM norvalin, store at below 8 °C not longer than 4 weeks		N/A
C.4.3	o-Phthaldialdehyde (OPA)		N/A
	50 mg o-phthaldialdehyde, 4,5 ml methanol, 50 µl mercaptopropionic acid		N/A
C.4.4	Boratebuffer		N/A
C.4.5	Stop-solution		N/A
C.4.6	Phosphate buffer		N/A
C.4.7	Solvent 1		N/A
	20 ml tetrahydrofuran plus 1 l phosphate buffer		N/A
C.4.8	Solvent 2		N/A
	250 ml acetonitril 100 ml tertrahydrofuran ad 1 l with phosphate buffer		N/A
C.4.9	Sodium carbonate solution (0,1 M)		N/A
	2,12 g Sodium carbonate in 10 ml water		N/A
C.5	Hydrolysis		N/A
C.5.1	400 µl extract (in TES buffer) + 10 µl norvalin-1+ 700 µl HCl		N/A
C.5.2	Standards		N/A
	380 µl water + 20 µl amino acid standard + 10 µl norvalin-1+ 700 µl HCl		N/A
C.5.3	Incubation (hydrolysis)		N/A
	ncubate samples and standards simultaneously for 48 h at 100 °C in sealed screw capped PP vessels. The vessels should be clamped into a screwed rack to avoid cracking of the caps. It is very important to hydrolyse standards and samples simultaneously in order to have equal temperature and time conditions.		N/A
	Cool down samples and standards, dry them in vacuum concentrator centrifuge or in a desiccator over NaOH or KOH in vacuum.		N/A

Clause(s)	Test(s)	Test Remarks	Result
	HCl need to be removed completely; otherwise the capacity of the borate buffer for derivatisation may be not sufficient.		N/A
C.5.4	Free amino acids		N/A
	Prepare from each extract and from the standard an un-hydrolysed sample.		N/A
	400 µl extract + 10 µl norvalin 1		N/A
	380 µl water + 20 µl amino acid standard+ 10 µl norvalin 1		N/A
C.6	Analysis (HPLC)		N/A
C.6.1	Sample preparation		N/A
	Add 20 µl sodium carbonate solution to the dried samples		N/A
	Mix well or sonicate.		N/A
C.6.2	Derivatisation		N/A
	The derivatisation step is dependent on time and temperature; it should be done by an autosampler at constant temperature between 20 °C and 25 °C.		N/A
	Mix 25 µl borate buffer, 12 µl OPA and 8 µl sample		N/A
	After 2.5 min terminate the reaction by adding 25 µl stop solution		N/A
C.6.3	HPLC		N/A
	The HPLC analysis can be done in any HPLC equipment using a gradient system and fluorescence detector A successful example is listed here, but these conditions have to be adapted to the system and the column used.		N/A
	0 min to 2,5 min — 0 % solvent 2 — 100 % solvent 1 2,5 min to 3,0 min — 0 % to 12,5% solvent 2 — 87,5 % to 100 % solvent 1 3,0 min to 9,0 min — 12,5 % solvent 2 — 87,5 % solvent 1 9,0 min to 13,0 min — 12,5 % to 42% solvent 2 — 58 % to 87,5 % solvent 1 13,0 min to 24,0 min — 42 % solvent 2 — 58 % solvent 1 24,0 min to 26,0 min — 42 % to 80% solvent 2 — 20 % to 58 % solvent 1 26,0 min to 30,0 min — 80 % solvent 2 — 20 % solvent 1 30,0 min to 31,0 min — 0 % to 80% solvent 2 — 20 % to 100 % solvent 1		N/A
C.6.4	Calculation		N/A
	The concentration of the individual amino acids shall be performed by an internal standard method with subtraction of the free amino acids. The sum of the amino acids equals the total protein content.		N/A

Photos

*******End of Test Report*******