Report n° CYTOTOX-PH-24/0344bis
Cytotoxicity screening assay using XTT Method

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Assay: The purpose of this study was to assess the cytotoxicity of a test item (extract) in an established cell line. This study will determine the *in vitro* biological response of mammalian cells using appropriate biological parameters.

The extract obtained from the test item (identification code: PH-24/0474) was tested to evaluate its ability to induce a cytotoxic effect in L929 (ATCC, CCL 1 NCTC clone 929) cells by measurement of viability using XTT (2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide) reagent.

Test item identification and characterization

Name:	ACCESS SOCKET TF TT BATCH OA ACCESS SOCKET
Surface:	75 cm²
Identification code:	PH-24/0344

Preparation of extract

Extraction vehicle:	Culture medium (DMEM) supplemented with 10% (v/v) FCS + 1% antibiotics
Extraction conditions:	Extraction temperature: 37°C ± 1°C - gentle agitation (8 rockings/minute) Extraction period: 24h Ratio between the surface area of the test material and the volume of extraction vehicle: 3 cm²/mL

Exposition of test item extract with cells

Cells were seeded at 30000 cells /cm² then incubated 24 hours at 37°C before exposition to test item extract. The 100% extract was incubated with cells at 37°C in a humidified atmosphere containing 5% (v/v) CO_2, for 24h. Negative control (complete culture medium) and positive control (Latex) were run in parallel (n=3 each condition).
After the 24 hours exposition cells were washed and exposed to XTT reagent during 4 hours at 37°C in a humidified atmosphere containing 5% (v/v) CO_2.
Viability was then quantitatively evaluated using a microplate reader (450 nm reading).

Results

Results are expressed as absorbance units (proportional to cell viability) and compared to negative control. Statistical analysis is performed using the Student's *t* test. P values less than 0.05 are considered statistically significant.
The result obtained for positive control validate the result obtained for the test item.

		Concentration	Absorbance (UA)	Viability	Student Test
Blank	**Extraction Vehicle**	**100 %**	1.4830 ± 0.0381	103 % **	-
Negative Control	**(Complete culture medium)**	-	1.4462 ± 0.0697	-	-
Absolute Negative Control	**(Complete culture medium without cells)**	-	0.2307 ± 0.0037	-	-
Positive Control	**Extract of LATEX**	**6 %**	0.2587 ± 0.0030	2 % *	P < 0.001
		4.5 %	0.2593 ± 0.0039	2 % *	P < 0.001
		3 %	0.5563 ± 0.0131	26 % *	P < 0.001
		1.5 %	1.4073 ± 0.0508	94 % *	P < 0.05
Extract of ACCESS SOCKET TF TT BATCH OA ACCESS SOCKET	**PH-24/0344**	**100 %**	1.4196 ± 0.0177	95 % *	P < 0.05

Tables results

* Versus blank
** Versus negative control
NS: not statistically significant (P ≥ 0.05)

Conclusion

Under the experimental conditions of this test, the extract obtained from the tested medical device ACCESS SOCKET TF TT BATCH OA ACCESS SOCKET provided by ORTHO ACCESS is not cytotoxic (a *test item (extract) is considered as cytotoxic if the inhibition of the cell growth is over 30%).*

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Please note screening analysis is not realized in compliance with GLP however adapted from SOP INT493 and NF EN ISO 10993-5 and NF EN ISO 10993-12