Cytotoxicity (ISO 10993-5)

Interlaboratory comparison - short report

1 Metainformation

1.1 Data protection, copyright and disclaimer

The distribution or reproduction of this interlaboratory comparison summary report is not permitted. All rights to the available data are held by the Johner Institute.

The focus in the evaluation of the results was on presenting the influence of as many test parameters as possible in favor of the highest possible information density, but on the other hand this had to be partially restricted to ensure that none of the participants could be identified.

For this purpose, no fewer than 3 laboratories were ever grouped together, and exact measured values are intentionally not shown in a readable form.

The evaluation of the interlaboratory comparison results therefore extends to the clear presentation of trends, especially when comparing the test parameters.

The Johner Institute assumes no liability for damages that should arise if a participant already has additional data from another source that makes another participant identifiable to him.

1.2 Data on the interlaboratory comparison

We were convinced that an interlaboratory comparison in the cytotoxicity test was possible. This may be less of an absolute comparison, but in any case, it can be a relative one with regard to the test setup and identical test setups amongst participants.

We are sure to generate added value for you as a testing laboratory conducting the interlaboratory comparison by assessing the quality of your test performance and estimating the sensitivity of your test system as compared to other laboratories. You also have a basis for comparison for future customer inquiries. There is huge benefit to the customer of being able to compare your tests with other approved suppliers.

More than 250 laboratories worldwide were invited for the present laboratory comparison 2021. A total of 56 laboratories participated in the interlaboratory comparison. Four of the participating laboratories had to be rated as "failed." The criteria for this was only the failure to submit the test results.

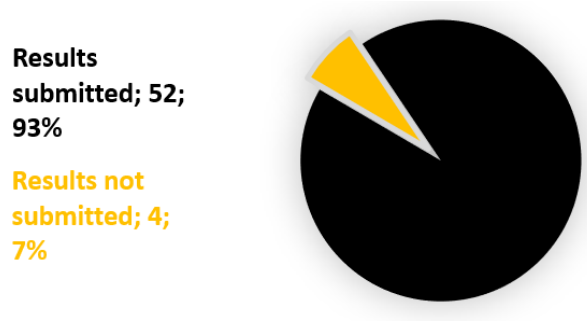


Fig. 1.2.1 Successful participation

The primary objective of the interlaboratory comparison is to compare laboratories with each other and not to determine the "repeatability" of the individual participant, therefore only a single determination was performed. We assume that the particular laboratory has already confirmed the repeatability of its test system.

1.3 Data on the test management

The Johner Institute has been supporting medical device manufacturers for many years in the development and approval of medical devices.

Our broad spectrum of technical experts allows us to act and think in an interdisciplinary way. However, we do not just see ourselves as a partner of medical device manufacturers - we have also set ourselves the goal of offering significant added value to the entire medical technology industry. In the area of biocompatibility, we are focusing on placing more emphasis on the necessity of testing at the lowest possible cost.

In the area of research and development, we are always looking for active partners and offer pro bono services in order to generate joint added value and a competitive edge for everyone. The same is true in this case.

Johner Institut
Villa Rheinburg
Reichenaustr. 1
78467 Constance
GERMANY

Contact details
+49 (7531) 94500 20
info@johner-institut.de
Website www.johner-institut.de

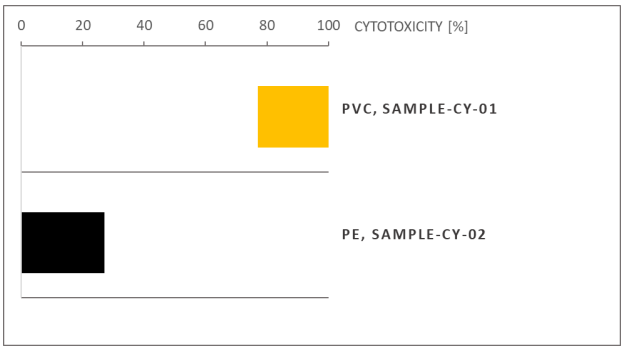
1.4 Data on the samples

2 materials were selected whose cytotoxic potential is generally known (may be assumed). First was polyethylene (PE) tubing, which can generally be assumed to be non-cytotoxic (inhibition of cell proliferation ≤ 30%) when properly manufactured and without residues. A polyvinyl chloride (PVC) tubing was also selected. Depending on the formulation, a cytotoxic potential can be expected for PVC materials (inhibition of cell proliferation > 30%).

Both materials were also selected for their high material quality (e.g. food grade), with the aim of eliminating natural possible variations in the material as far as possible. In addition, to further minimize such effects, for each sample, multiple samples were pooled (4 pieces of tubing as one sample per material).

Prior to the start of the interlaboratory comparison, one laboratory tested each of the materials for cytotoxicity ten times on different test days. It was shown that there are no significant material variations. There was a fluctuation range of approx. +/-17%.

Figure 1.5.1 shows these measured values. The bar represents the complete range of the measured values (range of the measured values).



Test Samples	Material	Mean (n = 10)	Min (n = 10)	Max (n = 10)
SAMPLE-CY-01	PVC	88,9	77	100
SAMPLE-CY-02	PE	11,5	0	27

Fig. 1.4.1 Overview of the test samples

The results are < 30% (non-cytotoxic) for PE and above 30% (cytotoxic) for PVC.

Due to the different test designs (all test designs of the participants who passed are normatively covered), larger variations and different sensitivities of the test systems were to be expected.

For this reason, hard limits cannot be set for test results. However, the measured values obtained above (and the expected values for the materials) serve as orientation.

Successful participation in the interlaboratory comparison must therefore not be assessed as a pass or fail across the board.

Due to different settings of the test and different methods of evaluation, cytotoxicity of < 0% and > 100% have been reported. All results were therefore set to 0% for values <0% and results >100% to 100% for better comparability. Furthermore, purely qualitative evaluations were converted to the corresponding cytotoxicity in percent based on the grade classification. Grade 0 ≙ 0%, grade 1 ≙ 10%, grade 2 ≙ 30%, grade 3 ≙ 60% and grade 4 ≙ 85%.

2 Results of the interlaboratory comparison

2.1 General results

The following graph (figure 2.1.1) shows all individual measured values for the PVC sorted in ascending order and the qualitative evaluation regarding cytotoxic (> 30% inhibition of cell proliferation) and not cytotoxic (≤ 30% inhibition of cell proliferation).

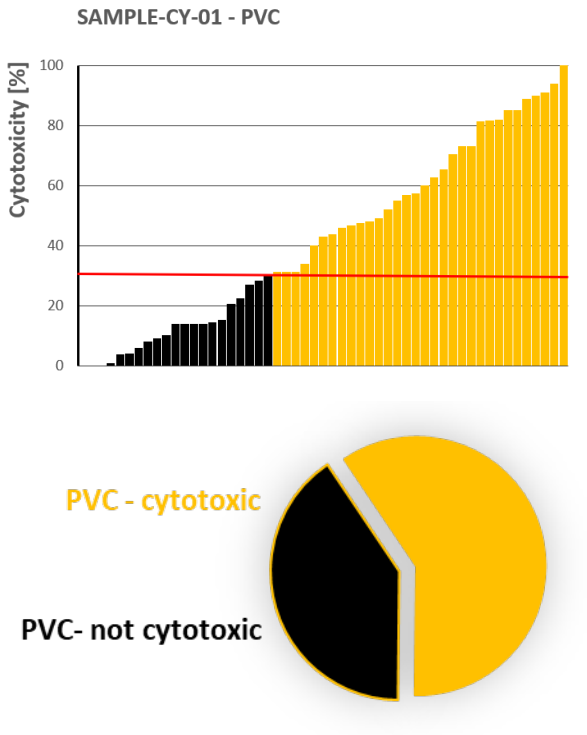


Fig. 2.1.1 Results of the PVC material in ascending order

It can be seen that from 0% to 100% cytotoxicity, all measured values occur with nearly equal probability. No clear tendency towards cytotoxicity is apparent.

Figure 2.1.2. shows all individual measurement values for the PE, in ascending order as well as the qualitative evaluation regarding cytotoxicity.

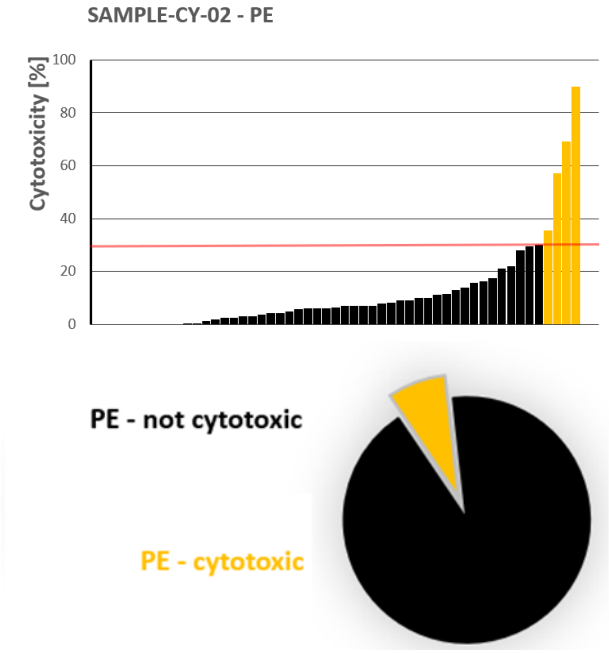


Fig. 2.1.2 Results of the PE material in ascending order

The measured values show no 100% clear result (not cytotoxic) for the PE material. However, most of the laboratories have even obtained values around 0-10 %.

It can therefore be summarized that different laboratories will generally not provide comparable measured values for an identical material or medical device.

Materials not classified as cytotoxic are expected to be identified as such for the most part. However, materials assumed to be cytotoxic are not identified as such by all laboratories.

Figure 2.1.3 shows the results for both materials in comparison. The results can be related using the connecting line for each laboratory.

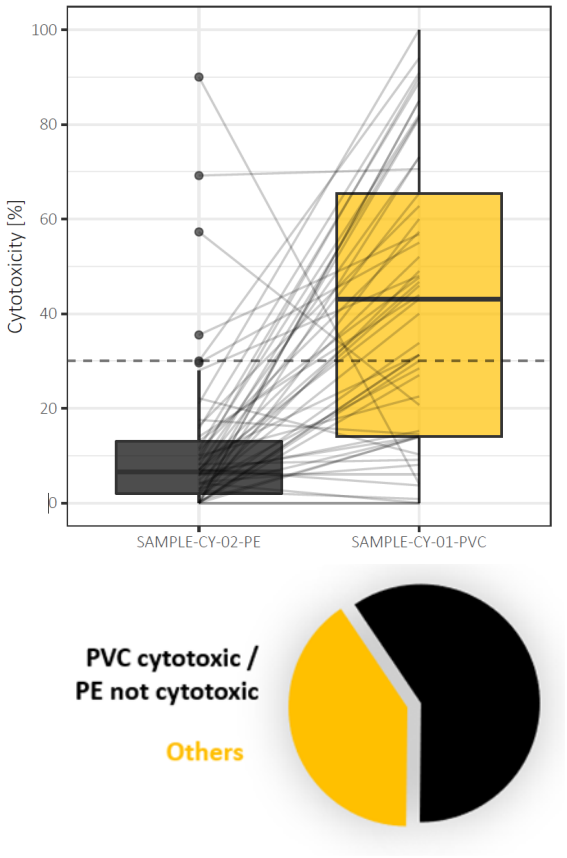


Fig. 2.1.3 Results of PVC and PE per laboratory

The comparison of the results per laboratory confirms the very different sensitivity of the test designs. All sensitivities and all conceivable gradations are present.

There is no consistent correlation between Figures 2.1.1 and 2.1.2. (it can therefore not necessarily be assumed that high measured values for PVC always require low measured values for PE). However, when PVC is evaluated as cytotoxic, the PE is generally classified as non-cytotoxic by more than 50% of the participating laboratories. The labs that classified PE as strong cytotoxic probably mixed up the samples (assumption based on the result of Fig. 2.1.3: high value of PE related to low value of PVC, with one exception).

2.2 Special results

The following graphs and diagrams are each composed of a quantitative representation (colored cuboids) and a box plot diagram.

The colored cuboids represent the relative number of laboratories with the corresponding test parameter. The box plot diagrams show bars in correlating color representing the range of the measured values, the median as a line and outliers at points.

It is valid for all graphs that even with obviously effective results, the influence of other, not evaluated parameters (e.g. plated cell number, handling of the samples when stopping the extraction etc.) cannot be excluded.

Furthermore, laboratories in the parameter groups "other" also submitted excellent test results (related to the expected value). The evaluation therefore does not represent a qualitative assessment.

Since no group comprises less than 3 laboratories, an assignment of the laboratories by test parameters is again not clearly possible.

Figure 2.2.1 shows the influence of serum concentration in the extraction medium on the result of the cytotoxicity test.

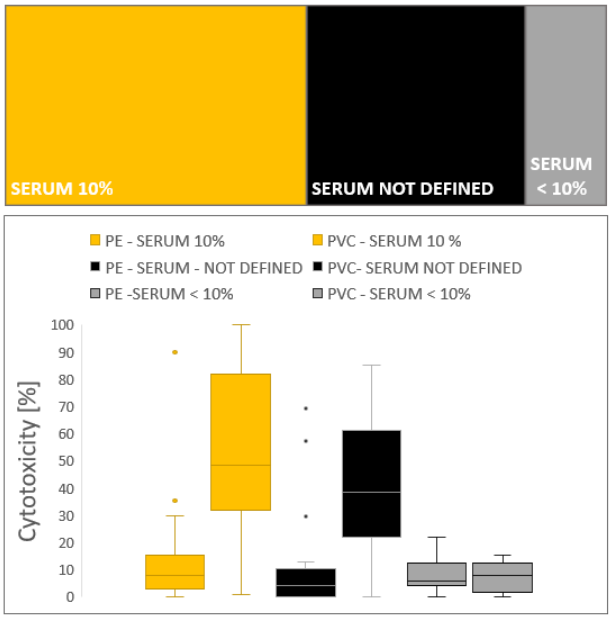


Fig. 2.2.1 Extraction (serum concentration)

The comparison clearly shows a significant influence of the serum concentration. Extraction medium with 10% serum content corresponds to the expected values for

the investigated materials. Serum concentrations less than 10 % provide the expected values for the non-cytotoxic material PE. However, the PVC material is not identified as expected, 100% of the PVC results is classified as non-cytotoxic, indicating poor sensitivity.

3. Discussion

3.1 General

It must be pointed out once again that in the interlaboratory comparison carried out, many parameters were specified which, under normal circumstances (this is confirmed by our experience over many comparable tests between individual laboratories), would be freely selected by the laboratory.

These include, for example, the extraction ratio, but also other extraction media, or extraction times. These parameters alone can have a massive impact on the final results. This means that the interlaboratory comparison scheme does not adequately represent the "normal commissioning" of a laboratory test.

For reasons of comparability, however, these specifications were made quite deliberately. It turns out that the reduction of the parameters was reasonable to reduce the amount of influences.

3.2 Other influencing factors

In the context of the interlaboratory comparison, it is also important to point out other influencing factors. Based on the test results, we assume that differences in handling, homogenization of extracts, extraction vessels, etc. had a significant effect on the results.

The aim of future interlaboratory comparisons in cytotoxicity testing should therefore be to synchronize the approximate test design with respect to further uncertainties and influencing factors.

3.3 Suggestions for the test setup

From our point of view, the available data, with slight limitations regarding the interpretation, nevertheless allow the unambiguous statement that individual parameters appear to be fundamentally very suitable for mapping the expected values, and thus the spectrum of non-cytotoxic to cytotoxic materials.

On the basis of the present evaluation (combination of most commonly used test parameters and most sensitive results) and our experience, we therefore take the liberty of making recommendations for optimizing the test design of the cytotoxicity test (ISO 10993-5). Table 1 demonstrates the suggested parameter (red) and an acceptable alternative parameter (black).

Table 1 Proposal for test design (red = preferred parameter, black = alternative parameter)

Medium	MEM + 10 % Serum DMEM + 10 % Serum
Incubation	24h pre-incubation, > 24h incubation 00h pre-incubation, 72h incubation
Assay	XTT BCA
Cells	L929

We hope that this will further harmonize the test design of the laboratories and thus generate added value for the medical device manufacturers through the comparability of results.

As already mentioned, the interlaboratory comparison is a support for classifying own test results in comparative tests of the medical device manufacturers. It also allows laboratories to align with your test systems on identified parameters that significantly impact the achievement of expected values.

Participation in the interlaboratory comparison is therefore seen as a free opportunity to scrutinize and optimize one's own test set-up and thus increase the quality of the results.

Dipl.-Ing. (FH) Sarah Gruber
Interlaboratory Comparison Management

Extended Version

In order to compensate the considerable costs for pre-tests, participant questions, test samples and working hours, we have considered to give you the possibility to provide an extended test report (8 additional graphs) with all possible evaluable test parameters for a small "cost contribution". If you do not want this, the participation is and remains free of charge for you, of course. Does this sound fair to you?

Your voluntary "cost sharing" motivates us to continue to offer proficiency testing free of charge. Simply write to us:

interlaboratory@johner-institut.de

Preview of the extended version report: