A novel bio-assay increases the detection yield of microbiological impurity of dialysis fluid, in comparison to the LAL-test

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Abstract

Background. Biological purity of dialysis water is considered as one of the primary conditions to deliver optimal haemodialysis.

Methods. The present study explores the added value of a novel cytokine (IL-1ß) induction assay, using a monocyteic THP-1 cell line, compared to the classical detection methods for microbial dialysis fluid contaminants.

Results. In contrast to the Limulus Amebocyte Lysate (LAL)-test, which only detects intact lipopolysaccharide (LPS), the THP-1 assay was also sensitive to peptidoglycan, short bacterial DNA fragments and LPS fragments <5 kD. The purity of 269 dialysis fluid samples was tested by the THP-1 assay and compared to the LAL-test. Two hundred and sixty samples complied with the definition of 'pure' dialysis fluid as laid down in the European Pharmacopeia (European Best Practice Guidelines for Hemodialysis. Section IV. Dialysis fluid purity. Nephrol Dial Transplant 2002; 17: 45–62) but 27 of these so-called pure dialysates (10.3%) provoked a pro-inflammatory response in the THP-1 assay. Furthermore, among the 230 samples that complied the definition of an ultrapure dialysis fluid, 21 samples (9.1%) were pro-inflammatory. These data illustrate that this novel bio-assay detects microbiological entities with an inflammatory potential that cannot be found by the classical LAL screening method.

Conclusions. Adding this novel THP-1 assay to the classical methods will be helpful in the prevention of biofilm formation in the delivery system and should have relevance by more accurate detection of dialysate contamination, hence decreasing micro-inflammation in the haemodialysis patient.

Keywords: bio-assay; dialysis fluid quality; haemodialysis; LAL-test

Introduction

The purity of dialysis fluids is of major importance in renal replacement therapy [1]. Contact of impure dialysate with the patient’s blood through semi-permeable membranes [2] contains a substantial risk for transfer of contaminants into the patient, potentially inducing an inflammatory reaction. This can be prevented by the preparation of ultrapure water; which becomes even more desirable with the application of high-flux or even albumin-leaking haemodialysis strategies and of on-line haemodiafiltration modalities [1,3].

A large variety of microbial contaminants (intact microorganisms and microbial degradation products and/or fragments) provoke inflammatory responses by stimulating leukocytes to produce pro-inflammatory cytokines [4–6]. Microbial fragments are released during growth and/or lysis of the microorganisms and have been shown to be present in dialysis fluids [5–7]. Even small fragments of bacterial DNA have been suggested to play a role in maintaining a chronic inflammatory state in haemodialysis patients by prolonging survival of inflammatory mononuclear cells [8].

The currently used and recommended methods for screening the purity of dialysis fluids do not guarantee the detection of all microbial contaminants. Many microorganisms in haemodialysis water are in a ‘viable but non-cultur able’ state, and their detection necessitates highly specific culture conditions, and/or imposes the need for more sophisticated molecular approaches [9]. Additional difficulties are encountered for the detection of microbial derivatives, although, in many cases, they may be the only traceable markers of contamination. For their detection, currently the Limulus Amebocyte Lysate (LAL)-test is recommended [1]. Nevertheless, intact LPS is the only one that can be detected by the LAL-test.

The timely detection of any kind of contaminant of the dialysis fluids is germane in order to avoid (1) biofilm formation in the dialysate delivery circuit and (2) micro-inflammation in the haemodialysis patient resulting in an enhanced risk for chronic morbidity complications [10].
The present study demonstrates the added value and in vivo relevance of a novel biological assay in the monitoring of the quality and biological activity of dialysis fluids. Using a cytokine (IL-1β) induction assay in a monocytic THP-1 cell line we explored whether the presence of different types of microbial fragments, undetectable with the LAL-test, could be traced.

Subjects and methods

Materials

The following materials were used in the study: peptidoglycan (PGN) from S. aureus (Fluka, Buchs, Switzerland); short bacterial DNA fragments (oligodeoxynucleotides, ODN) (TIB MOLBIOL, Berlin, Germany); Pseudomonas aeruginosa (LMG 8029, Gent, Belgium); lipopolysaccharide (LPS) from Escherichia coli 0111:B4 (Sigma-Aldrich, St Louis, MO, USA); sodium chloride solution (0.9%) (Baxter, Lessines, Belgium) used to dissolve PGN, ODN, LPS and the LPS fragments; Dulbecco’s PBS 10× (DPBS, lowest available level of endotoxin) (Gibco, Invitrogen, Paisley, UK).

Whole blood collection and cell cultures

Heparinized whole blood from healthy volunteers was collected (BD Vacutainer systems, Plymouth, UK). The number of leukocytes was counted (Coulter Counter, Luton, England), and the percentage of mononuclear cells was determined by flow cytometry (FACScan™, Becton Dickinson, San Jose, CA, USA).

The human monocytic cell line THP-1 (ATCC, LGC Promochem, Middlesex, UK) was maintained as a continuous culture.

LAL assay

The LAL kinetic chromogenic assay (Kinetic-QCL®) (Lonza, Walkersville, MD, USA) detects intact LPS with a detection limit of 0.005 endotoxin units (EU)/ml.

Detection of oligonucleotides

Oligonucleotides were detected by using the Quant-it OligoGreen kit (Molecular Probes, Eugene, OR, USA). Black opaque stripwell 1 × 8 strips and frames were procured from Costar (Cambridge, MA, USA). The linearity and detection limits of the OligoGreen ssDNA assay were determined by making a series of dilutions ranging from 0 to 50 ng/ml using a Tris–EDTA (TE) buffer (10 mM Tris, 1 mM EDTA, pH 7.5) as the diluent. After aliquoting 100 µl of the samples or standards, 100 µl of OligoGreen working solution was added to each well. The samples were incubated for approximately 5 min at room temperature and their fluorescence determined using a FLUOtest Optima fluorescent plate reader with a 480 nm excitation filter and a 520 nm emission filter. The sensitivity of the assay was 1250 pg/ml.

Silkworm larvae plasma (SLP) test

PGN concentration was estimated with the silkworm larvae plasma test (Wako Pure Chemical Industries, Osaka, Japan) according to the manufacturer’s guidelines. The limit of detection of PGN was 7.8 µg/l.

Test solutions

PGN was dissolved and diluted in saline. The effect of PGN was tested in a dose-response set-up (0.1; 1; 5; 10; 50; 100; 500; 1000 and 5000 ng/ml).

The three oligonucleotide stock solutions (2006 stimu, 2006 Flip and K3) were diluted in saline resulting in 0.1, 1 and 10 µM concentrated solutions. The oligodeoxynucleotide 2006 stimu [(24 mer): TCGTGTGTTTGTGTTTTGTTGTCGTT] and the K3 [(20 mer): ATCGACTCTCGAGCGTTCTC] ODN are CG-rich fragments, which have been identified as pro-inflammatory [11,12], while 2006 Flip [(24 mer): TGCTGTTTTGTTGTTTGTTGTT] served as a non-inflammatory CG-lacking control.

LPS fragments were obtained after a 15-min sonication period of an active P. aeruginosa culture followed by filtration through a 0.22 µm filter (Millex-GV, Millipore, Carrigtwohill, Ireland) and subsequently through a regenerated cellulose filter with a cut-off of 5000 Da and a low adsorptive capacity (Amicon, Ultracel 1000, Millipore, Bedford, MA, USA). The solutions obtained this way were tested as such, without further treatment or dilution.

LPS (intact) from E. coli was dissolved and diluted in saline and also tested in a dose-response setting (0.005; 0.01; 0.1; 1; 10; 100 ng/ml).

As iv pharmacological control solution, the isotonic sodium chloride solution (0.9%) (Baxter, Lessines, Belgium), which was used as solvent for the test solutions, was included per se. Repetitive experiments (n = 6) were performed on separate days.

Cytokine induction assay

Whole blood. The cytokine induction assay using heparinized whole blood was applied as described in 1998 by Lonnemann [13]. In brief, whole blood was incubated in a 1:1 dilution (with a total volume of 700 µl) with the respective test solutions or dialysis fluids in polystyrene pyrogen-free culture plates (Nunc, Roskilde, Denmark) for 24 h in a humified atmosphere of 5% CO2 in air at 37°C. After incubation, the whole blood culture supernatant was collected after centrifugation for 10 min at 1000 g. Supernatants were stored at −20°C until cytokine analysis.

Repetitive experiments (n = 6) were performed using separate donors.

THP-1 cell culture. Cell cultures (1 × 106 cells/ml) were treated for 72 h with calcitriol (10 nM) (a gift from Roche Pharmaceuticals, Basle, Switzerland) after which the medium was refreshed followed by a 24-h rest period before use in the cytokine induction assay as described above.


**IL-1β detection assay**

Conforming to the Lonnemann cytokine induction assay [13] and because Gram-positive and Gram-negative bacteria were shown to equally induce IL-1β in contrast to TNF-α e.g. [4], IL-1β was quantified. The supernatant concentrations of IL-1β were measured using a sandwich ELISA kit (Quantikine R&D Systems, Abingdon, UK). The minimum detectable dose of IL-1β is 1 pg/ml. IL-1β levels were normalized to an identical number (1 x 10⁹/ml) of mononuclear cells (MNC) in whole blood and THP-1 cells (in cell cultures).

**Sampling**

To evaluate the adequacy of this test method, it was applied to test the response of dialysate samples collected at our own unit (n = 112) and 30 different external units (n = 157) throughout the world [Belgium (15 units), Tunisia (3), Turkey (4), Lithuania (5), USA (New York (3))].

Sampling occurs with a sterile needle and syringe under aseptic conditions using 0.5% chlorhexidine/60% isopropanol disinfection of the sample port allowing the alcohol to evaporate before sampling. Samples are processed immediately after sampling.

In the external units, when sampling ports were available, the same sampling procedure was followed as described above. In case no sampling ports were available, samples were collected after discarding the first 100 ml of outflowing dialysate.

The samples collected in Belgian dialysis units were immediately transported at 4°C to our laboratory and were analysed within 24 h after sampling time, while the samples from abroad were immediately frozen, preferably at −80°C. They were sent to our laboratory on dry ice. All samples were collected in the same 15 ml pyrogen-free polystyrene tubes (Sarstedt, Nümbrecht, Germany) that were sent to the contributing units before collection together with a standard sampling protocol.

In addition, 44 isotonic sodium chloride solutions (0.9%) (Baxter, Lessines, Belgium) were sampled according to the above-described protocol, using the same syringes, collection tubes, storage and transportation conditions.

For the present study, application of the THP-1 cytokine induction assay was performed in parallel with the LAL-test. A 10 × DPBS solution was added (10:1) to the dialysis water samples to induce isotonicity of the solution, needed to preserve leukocyte viability during the cytokine induction assay. At each test, three control samples were evaluated, two negative controls [(1) cell culture medium per se and (2) LAL-free water with 10 × DPBS] and one positive control (2) with 50 EU/ml LPS. A sample was considered to be biologically active when the cytokine secretion was more than two times the cytokine secretion of the negative control samples.

**Statistical analysis**

Data are expressed as mean ± SD. Statistical analysis was performed using a paired Wilcoxon signed rank test or an unpaired t-test (Table 2) using GraphPad Prism 4.0. To statistically assess the difference in variance between the

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**Results**

**Response to different types of contaminants**

The first part of this study compares the biological response towards various types of contaminants by the THP-1 test, which is the focus of this publication, to (1) the LAL-test and to (2) a biological assay based on whole blood from healthy volunteers.

**Peptidoglycan.** The THP-1 assay was more sensitive to PGN than the whole blood assay, with a significant induction of IL-1β secretion, compared to saline, from a concentration of 5 ng PGN/ml on (Figure 1). In the whole blood assay, a significant increase in IL-1β secretion was observed from 500 ng PGN/ml on (P < 0.05 versus THP-1). For PGN concentrations above 500 ng/ml, the variance (modified Levene test) was significantly more pronounced in the whole blood assay compared to the THP-1 assay (P < 0.05 versus THP-1). In addition, THP-1 cells secreted significantly more IL-1β in the presence of 5 to 1000 ng PGN/ml compared to whole blood leukocytes. For each of the tested PGN concentrations, the classical LAL-test remained unresponsive (<0.005 EU/ml).

**Short bacterial DNA fragments or oligodeoxynucleotides (ODN).** The stock solutions of the three different ODN were diluted to obtain 0.1, 1 and 10 μM test solutions.
First, the reactivity of each test solution was checked in the LAL assay, resulting in no response for the 0.1 and 1 µM solutions, and only a minor response corresponding to an LPS concentration of 0.013 ± 0.001 EU/ml in all the 10 µM concentrated solutions, but this concentration did not show any cytokine induction capacity (data not shown). As illustrated in Figure 2, only oligodeoxynucleotide 1 from a concentration of 1 µM on and oligodeoxynucleotide 3 from a concentration of 10 µM significantly induced IL-1β secretion in the THP-1 assay, compared to saline control. In the whole blood assay, only oligodeoxynucleotide 3 at 10 µM caused a minimal degree of cytokine induction. *P < 0.05 versus 0; **P < 0.05 versus THP-1; n = 6.

**LPS fragments (<5 kD)**. The filtrate from a *Pseudomonas aeruginosa* (*P. aeruginosa*) culture containing LPS fragments showed a significant induction of IL-1β versus control (60.7 ± 22.5 versus 1.7 ± 0.7 pg/ml, P < 0.05) in the THP-1 assay, but this sample showed no response in the LAL-test. Although the mean biological response in the whole blood assay was higher on the average (140.9 ± 144.4 pg/ml) compared to the response in the THP-1 assay (60.7 ± 22.5 pg/ml), the mean response with both test methods was not significantly different. The higher response in whole blood was essentially attributable to a larger donor-related variability in comparison to the THP-1 assay as proven by the significant modified Levene test (P < 0.05) (Figure 3). Figure 3 shows that when applying the whole blood assay, detection of contamination with LPS fragments could be overlooked or at least strongly underestimated due to the possibility of an insufficiently pronounced response by specific donors, as two of six donors show a response close to zero.

**Intact LPS**. Figure 4 illustrates that the sensitivity of the cytokine induction assay using THP-1 cells is similar to the sensitivity of the assay using whole blood, both showing a significant induction of IL-1β secretion from an LPS concentration of 0.1 ng/ml on. It is of note that the overall variability was more pronounced in the whole blood cytokine induction assay compared to the THP-1 assay. Each of the tested LPS solutions resulted in a positive LAL-test confirming the pursued concentrations.
Application of the THP-1 cytokine induction assay to collected dialysis fluid samples

In the second part of this study, a comparison between the response in the THP-1 IL-1ß assay and the LAL-test was performed on 269 dialysis fluid (43 water/226 dialysate) samples and 44 control saline samples.

All control samples showed a response below detection limit (<0.005 EU/ml) in the LAL-test, together with a response equal to the background response of 6.9 ± 2.0 pg IL-1ß/ml in the THP-1 assay.

From the total of 269, 260 (water/dialysate) samples complied with the definition of pure dialysis fluid (<0.25 EU/ml) according to the European Pharmacopeia. Nevertheless, 27 of these ‘pure’ dialysates (10.3%) provoked a pro-inflammatory response with the THP-1 assay. Furthermore, among the 230 of the samples that complied with the definition of ultrapure dialysate fluid (<0.03 EU/ml) according to the European Pharmacopeia or of dialysate for infusion according to the Association for the Advancement of Medical Instrumentation (AAMI), 21 samples (9.1%) induced IL-1ß secretion in the THP-1 assay.

A substantial number (10.3%) of biologically active dialysis fluid samples were detected by the THP-1 assay that would have been missed with the LAL-test. In addition, 9.1% of the samples categorized as ultrapure by the LAL-test also induced IL-1ß activity.

Table 1 illustrates that the mean IL-1ß secretion in the ‘pure’ samples is significantly higher than the one expected from the LPS content as quantified by the LAL-test. Nine of the tested dialysate samples had an endotoxin content above 0.25 EU/ml (1.38 ± 2.37 EU/ml) and were classified as ‘impure’ but here also the mean IL-1ß inducing capacity was three times higher than the one expected from the measured LPS concentration (real: 72.5 ± 86.3 versus expected: 21.7 ± 25.6 pg/ml). The significant difference between the measured and the expected IL-1ß secretion, as shown in Table 1, indicates that these dialysate samples likely contain other microbiological derivatives than LPS (or other LPS than from E. coli) so that their cytokine induction capacity is insufficiently reflected by the LAL-test.

Table 1. Response of dialysis fluid samples with a different purity grade in the LAL-test compared to the THP-1 assay

<table>
<thead>
<tr>
<th></th>
<th>Pure (n = 260)</th>
<th>Impure (n = 9)</th>
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<tbody>
<tr>
<td>LAL (EU/ml)</td>
<td>0.11 ± 0.07</td>
<td>1.38 ± 2.37</td>
</tr>
<tr>
<td>IL-1ß (expected) (pg/ml)</td>
<td>7.91 ± 0.76</td>
<td>21.71 ± 25.65</td>
</tr>
<tr>
<td>IL-1ß (measured) (pg/ml)</td>
<td>35.23 ± 89.11**</td>
<td>72.49 ± 86.27*</td>
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</table>

**P < 0.001, *P < 0.05 versus IL-1ß (expected).

Receptor operating characteristics (ROC) analysis

The ROC curve for the THP-1 assay has an AUC of 0.810 ± 0.032 (95% confidence interval: 0.746–0.873; P < 0.0001) and is illustrated in Figure 5. This demonstrates that the THP-1 assay has high discriminative power. The corresponding ROC graph for the LAL-test has an AUC of 0.682 ± 0.040 (95% confidence interval: 0.603–0.761; P = 0.0003).

Fig. 5. Receiver operating characteristics (ROC) curve for the THP-1 assay (controls: n = 44, samples n = 269). Area under the curve (AUC): 0.810 ± 0.032 (95% confidence interval: 0.746–0.873; P < 0.0001).

Table 2. Cytokine induction capacity and oligonucleotide content in the samples containing <0.25 EU/ml

<table>
<thead>
<tr>
<th></th>
<th>LAL (EU/ml)</th>
<th>IL-1ß (pg/ml)</th>
<th>ODN (ng/ml)</th>
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<tr>
<td>(A) IL-1ß negative samples (n = 109)</td>
<td>0.014 ± 0.035</td>
<td>11.1 ± 5.3</td>
<td>1.6 ± 1.1</td>
</tr>
<tr>
<td>(B) IL-1ß positive samples (n = 16)</td>
<td>0.045 ± 0.072**</td>
<td>91.3 ± 112.8**</td>
<td>2.2 ± 1.8*</td>
</tr>
</tbody>
</table>

**P < 0.01, *P < 0.05 versus A.

Reproducibility of the THP-1 results

Four samples (3 positive and 1 negative in the THP-1 assay), which had been aliquoted in sterile polypropylene microtubes (Sarstedt, Nümbrecht, Germany) and stored at −20°C, were reanalysed on 5 different days revealing a variation coefficient between 9.17% and 19.4%. Positivity (for the three positive samples) and negativity (for the negative sample) was repetitively found.

Evaluation of the cytokine induction capacity of dialysate samples containing <0.25 EU/ml

Table 2 shows that the oligonucleotide concentration in the dialysate samples containing <0.25 EU/ml (n = 125) but with the capacity to induce IL-1ß secretion (n = 16) is significantly higher compared to the dialysate samples without IL-1ß induction capacity (n = 109). Three ODN samples were negative among the THP-1 positive samples and 88 ODN samples were negative among the THP-1 negative samples (n = 109). Note that ODN negativity means <1.25 ng/ml (detection limit); some of these samples can still contain ODN, but below detection limit, which can play a role in the cumulated biological activity added up to that of other microbial derivatives.

Only three dialysate samples contained PGN as shown in Table 3. Of note, all three samples induced IL-1ß secretion, and detection of biologically active contaminants present in sample 2 would not have been possible by only performing the LAL-test. Table 2 also demonstrates that co-stimulation might play an important role in the cytokine induction capacity of the samples. The concentration of one single contaminant, per se, might be too low to induce cytokine secretion; nevertheless, its biological effect will be...
amplified in the presence of other types of contaminants. As illustrated in Table 3, all three samples contain at least two (sample 3) of the three evaluated types of microbial derivatives, whereas samples 1 and 2 contain all three of them.

**Discussion**

The present study demonstrates that, compared to the LAL-test and the whole blood IL-1β assay, the THP-1 IL-1β assay is more sensitive to most currently known biologically active pro-inflammatory microbial derivatives. Biological purity of dialysis water is one of the primary conditions to deliver optimal haemodialysis [1]. Several international institutions and/or workgroups [1,14] developed recommendations for maintaining optimal purity of dialysis water (http://www.kdigo.org). According to the European Best Practice Guidelines for dialysis fluid purity, contemporary haemodialysis requires the use of water complying at minimum with the recommendations of the European Pharmacopoeia: bacterial count <100 Colony Forming Units (CFU)/ml and endotoxin content <0.25 EU/ml. However, for conventional and high-flux haemodialysis, the use of ultrapure water (bacterial count <0.1 CFU/ml and endotoxin content <0.03 EU/ml) is strongly recommended [1].

One of the major concerns is that biological impurities are prone to activate leukocytes and certain tissue cells such as endothelial cells, hence inducing overt pyrogenic reactions or more likely chronic and hidden micro-inflammation [15]. The latter is held responsible for a host of side effects [16–21], which all impact on the survival of dialysis patients [22]. Up till now, no large randomized clinical trials have evaluated whether ultrapure dialysate significantly reduces biomarkers of inflammation and other putative chronic consequential complications [23]. It cannot be denied that water purity has become a more stringent necessity since the introduction of large pore dialyzers that necessitate water purity has become a more stringent necessity since the introduction of large pore dialyzers that have been introduced with the intention to optimize convection and removal of uraemic retention solutes with a higher molecular weight [3] that have been linked to clinical complications of uraemic retention [24] and outcome of dialysis patients [25,26]. As a counterpart of this removal of large molecules, however, in the case of dialysate containing biologically active bacteriological compounds, transfer of pro-inflammatory contaminants into the blood compartment may be expected due to a favourable dialysate to blood concentration gradient.

Bacteriological contamination may be detected by growing water bacteria if appropriate culture conditions are applied [1,9]. Even while applying the most stringent methodology, intact bacteria remain unrecognized, if they desintegrate before they can be cultured, or if they remain entangled within the water delivery system e.g. in the case of biofilm formation in the piping system and/or filters. Therefore, the guidelines give threshold recommendations not only for bacteriological contamination but also for bacteriological pro-inflammatory secretion products [1]. Unfortunately, all guidelines restrict their instructions to intact lipopolysaccharides (LPS) by recommending threshold levels for the LAL-test. Intact LPS is a typical derivative of Gram-negative bacteria such as *E. coli*, but infectious dialysis water contaminants can also contain entities that are not detected by the LAL-test, e.g. LPS-fragments produced by Gram-negatives such as Pseudomonas [5]. Furthermore, Gram-positive bacteria also release bio-active oligodeoxynucleotides [6]. For all those reasons, microbial contamination might go unrecognized even if the detection methods propagated by the major guidelines are applied punctually and strictly.

Lonnemann *et al.* developed a biological test for the detection of bacterial contaminants other than intact LPS, based on the inflammatory response of whole blood from healthy volunteers [13]. The presently described method extends the possibilities described above by (1) omitting the need for healthy donors, being based on the use of a cultured monocytic cell line rather than blood cells from healthy volunteers; (2) increasing the sensitivity [27] to microbial derivatives such as PGN and ODN (Figures 1 and 2); (3) and (3) decreasing the test-related variance in response to PGN, LPS fragments <5 kD and intact LPS (Figure 1, 3 and 4). Note that for future experiments, the sensitivity of the assay can be improved by lysing the cells before collection of the supernatant for IL-1β quantification as described by Lonnemann [28].

Applying our test method to 269 samples of water and dialysate collected world-wide, it turned out that 10.3% of those categorized as pure, and 9.1% of those categorized as ultrapure induced IL-1β activity, whereas for all conditions considered, biological activity was more substantial than what could be extrapolated from the LPS concentrations estimated by the LAL-test (Table 1). Further analysis of the samples that were positive in the THP-1 test revealed more contamination with ODN (Table 2), and the presence, in some of these samples, of PGN sometimes in combination with significant amounts of ODN (Table 3). Our data underscore the complexity of clinically significant bacteriological contamination of dialysate, if the screening possibilities are expanded beyond the LAL-test.

The main purpose of the test method described is to detect microbial contamination of the dialysis water early during its development, and even more importantly, in direct correlation with its biological relevance, i.e. the capacity to induce inflammatory response in leukocytes. Subsequent to the finding of activity, the necessary decontamination measures can be taken. The application of this assay will be useful to reveal contaminants that otherwise go undetected, aiming at the timely prevention of biofilm formation in the dialysis circuit and micro-inflammation in the haemodialysis patient.

**Table 3.** Microbial derivatives present in the peptidoglycan containing samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>LAL (EU/ml)</th>
<th>ODN (ng/ml)</th>
<th>PGN (ng/ml)</th>
<th>IL-1β (pg/ml)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0.60</td>
<td>10.1</td>
<td>20.0</td>
<td>224</td>
</tr>
<tr>
<td>2</td>
<td>0.02</td>
<td>7.1</td>
<td>9.0</td>
<td>62</td>
</tr>
<tr>
<td>3</td>
<td>7.68</td>
<td>&lt;1.2</td>
<td>13.0</td>
<td>261</td>
</tr>
</tbody>
</table>
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