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# Collaborative Study of Bacterial Endotoxins Test Using Recombinant Factor C-Based Procedure for Detection of Lipopolysaccharides (Part 3)

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**Summary**

Two new recombinant cascade reagents (rCRs), PyroSmart NextGen® and PYROSTAR™ Neo, have recently become available for endotoxin testing of parenteral drugs in the Japanese market. This study investigated whether these two rCRs, as well as the current commercially available recombinant factor C reagents (rFCs) PyroGene™ and EndoZyme® II, can be used as alternative reagents to the amoebocyte lysate reagents currently used in the compendial Bacterial Endotoxins Test. The two rFCs were investigated in the previous two-year study.

An *Escherichia coli* O113: H10: K negative culture supernatant and seven water samples (six different tap waters and one deionized water) were tested for autochthonous endotoxin, and the endotoxin levels detected with four amoebocyte

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lysates and four recombinant protein reagents were compared. The results indicate that the four recombinant protein reagents can detect autochthonous endotoxin in culture supernatant samples at levels comparable (within the 50%-200% range as defined in the Pharmacopeias) to those measured with limulus amoebocyte lysate reagents. One of the four recombinant reagents detected autochthonous endotoxin in water at comparable levels to those obtained with lysate reagents in all samples, whereas the other three reagents detected comparable or lower levels among different samples. These findings suggest that there are differences in the detectability of autochthonous endotoxin in water among the recombinant protein reagents.

**Key words**

Bacterial endotoxin test, Amoebocyte lysate reagent, Recombinant factor C reagent, Recombinant cascade reagent

## 1. Introduction

Endotoxin (also referred to as lipopolysaccharide, LPS) in the cell walls of Gram-negative bacteria is a strongly pyrogenic substance that can activate innate immune responses[1]. Trace amounts of endotoxin directly invading the bloodstream can induce fever, and larger amounts can lead to life-threatening septic shock. Therefore, methods to detect endotoxin contamination in products are critical. The Bacterial Endotoxins Test (BET) is listed in chapter 4.01 of the Japanese Pharmacopoeia eighteenth edition (JP 18)[2] as a method to test endotoxin contamination in parenteral drugs.

The BET harmonized among the JP, the European Pharmacopoeia (Ph. Eur.)[3] and the United States Pharmacopeia (USP)[4] is a method to detect or quantitate endotoxin using amoebocyte lysate reagent (*Limulus* amoebocyte lysate, LAL or *Tachypleus* amoebocyte lysate, TAL) prepared by extraction of hemocytes from horseshoe crabs (*Limulus polyphemus* or *Tachypleus tridentatus*). The BET has three different methodologies: one is the gel-clot assay, which involves visual observation of gel formation; the second is a turbidimetric assay that measures the time required to reach a threshold optical density caused by changes in turbidity; and the last is a colorimetric assay, which measures optical color development resulting from cleavage of a synthetic substrate from a peptide.

Over the last several years, rFCs comprising only recombinant factor C, an endotoxin-sensitive serine protease zymogen, have been developed and introduced to the market to aid in the conservation of the horseshoe crab, provide a stable supply of reagent, and improve the consistency of testing results by eliminating the biological lot-to-lot variability of amoebocyte lysate reagents[5]. Lonza and Hyglos/bioMérieux developed and introduced the recombinant protein reagents, PyroGene™ and EndoZyme®, respectively, to the market. Each reagent contains a single recombinant zymogen protease (factor C) from horseshoe crabs. PyroGene™ has factor C from *Carcinoscorpius rotundicauda*, whereas EndoZyme® contains *T. tridentatus* factor C[6]. Recently, a recombinant cascade reagent (rCR), PyroSmart NextGen®, comprising rFC, recombinant factor B and recombinant proclotting enzyme, was developed and introduced to the market by Associates of Cape Cod, Inc. and Seikagaku Corporation[7]. This was followed by another rCR, PYROSTAR™ Neo, from FUJIFILM Wako Pure Chemical Corporation. Both rCRs use recombinant factors from *L. polyphemus*. Cascade reactions of amoebocyte lysate (panel A) , rFC (panel B) and rCR (panel C) reagents in the presence of endotoxin are shown in Fig. 1. These recombinant protein reagents cannot be used for BET as described in the Pharmacopeias, since an amoebocyte lysate prepared from blood corpuscle


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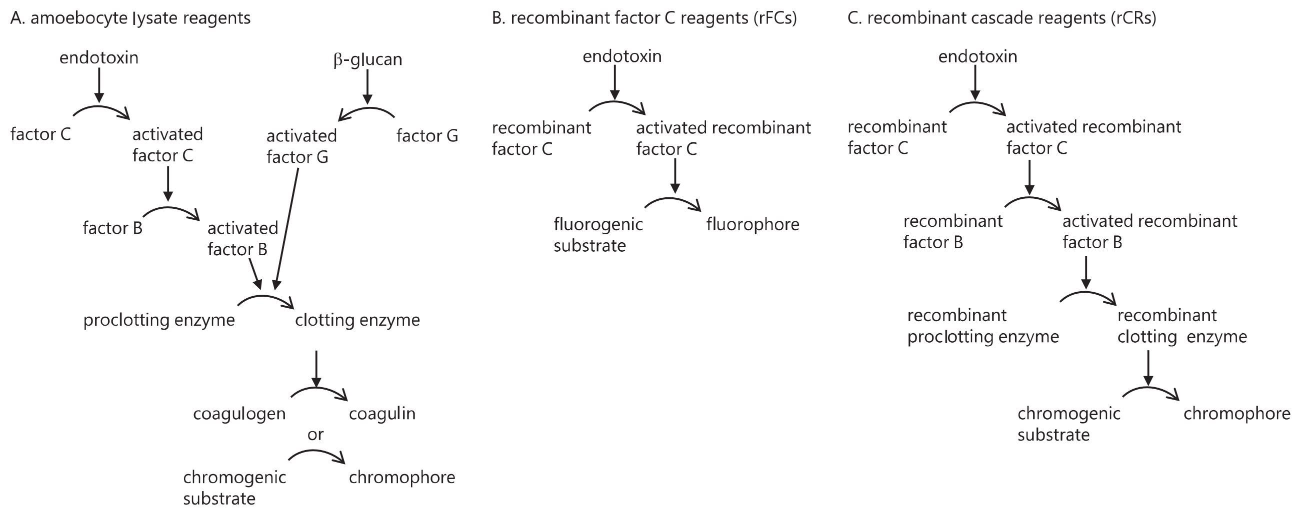


Fig. 1 Cascade Reactions of (A) Amoebocyte Lysate, (B) Recombinant Factor C and (C) Recombinant Cascade Reagents.

extracts of horseshoe crabs (*L. polyphemus* or *T. tridentatus*) must be used[2]. However, the US Food and Drug Administration (FDA)[8] states that rFC-based procedures can be used to test endotoxin as an alternative method to the BET[4] after validation in accordance with USP Chapter <1225> Validation of Compendial Procedures[9]. The Ph. Eur. also states that rFCs can be used as compendial methods after validation[10]. In June 2021, the JP 18 issued Bacterial endotoxins test and alternative methods using recombinant protein-reagents for endotoxin assay <*G4-4-180*>[11] as General Information, permitting alternative methods to be adopted after validation. However, in Japan validation of recombinant protein reagents to replace amoebocyte lysate reagents has so far been limited due to lengthy discussions surrounding different approaches for implementing alternative analytical procedures by end-users and manufacturers[7, 12~16].

So far, the appropriateness of using recombinant protein reagents to test endotoxin as alternative methods to the BET has been evaluated in two studies in 2017[17] and 2018[18]. Those studies concluded that recombinant protein reagents have lot-to-lot consistency and similar performance relative to amoebocyte lysate reagents when testing the reactivity to LPS purified from several different species of bacteria as well as autochthonous endotoxin [called naturally occurring endotoxin (NOE) in the study reports].

In this study, the performance of the two newly available rCRs in Japan as well as the existing rFCs was compared to that of four amoebocyte lysate reagents for the detection of autochthonous endotoxin in water, which is used as a raw material for parenteral drugs, and in culture supernatant of *E. coli* O113: H10: K negative, which is the same bacterial strain as that used to provide a reference standard endotoxin by the JP, USP and Ph. Eur. Furthermore, *E. coli* O113: H10: K negative is also used by the World Health Organization (WHO) as an international reference standard.

## 2. Materials and Methods

### Apparatus

All glassware was washed and then depyogenated in a hot-air oven using a validated process. Plastic materials, such as multi-well plates and tips for micropipettes, were used after they had been determined to be free of detectable endotoxin and to cause no interference with the


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test.

### Endotoxin

JP-Reference Standard Endotoxin (JP-RSE) was purchased from the Pharmaceutical and Medical Device Regulatory Science Society of Japan (PMRJ, Osaka, Japan).

### Water samples

Six tap water samples and one deionized water sample were collected from the seven institutes that participated in this study. After collection, each water sample was aliquoted into tubes, and the aliquots were kept frozen at -80℃ until use.

### Preparation of culture supernatant of *Escherichia coli* O113: H10: K negative

*E. coli* O113: H10: K negative was cultured according to the reported method[19] with some modifications. Briefly, the culture medium was a minimal microbial growth medium containing sodium phosphate (dibasic), monopotassium phosphate, sodium chloride, ammonium chloride, glucose, magnesium sulfate and calcium chloride. After culturing, the medium was centrifuged at 2,330 × *g* for 5 min at 4℃ to separate the culture supernatant. The supernatant was sterilized by filtration through a membrane filter with a pore size not exceeding 0.22 μm (Merck code # S2GPU05RE, Darmstadt, Germany) and kept frozen at -80℃ until use.

### Amoebocyte lysate reagents

Endochrome-K™ with endotoxin-specific reconstitution buffer was purchased from Charles River Laboratories (MA, USA). Endospecy® ES-50M was purchased from Seikagaku Corporation (Tokyo, Japan) and Kinetic-QCL™ was purchased from Lonza (MD, USA). PYROSTAR™ ES-F was purchased from FUJIFILM Wako Pure Chemical Corporation (Tokyo, Japan).

### Recombinant protein reagents

As rFCs, EndoZyme® II was purchased from Hyglos/bioMérieux (Munich, Germany) and PyroGene™ was purchased from Lonza (MD, USA). As rCRs, PyroSmart NextGen® was purchased from Associates of Cape Cod, Inc. (MA, USA) and PYROSTAR™ Neo was purchased from FUJIFILM Wako Pure Chemical Corporation (Tokyo, Japan). PyroSmart NextGen® was used both in onset time assay, which measures the time required to reach a threshold absorbance, and rate assay, which measures the mean rate of color development, unless otherwise mentioned.

### Endotoxin Assay

Endotoxin in the water samples and culture supernatant was measured using amoebocyte lysate and recombinant protein reagents. All reagents were used according to their Instructions for Use (IFU). JP-RSE was employed as a standard, and three two-fold dilution series were prepared with the following concentrations: 0.125, 0.0625, 0.03125, 0.015625 and 0.0078125 endotoxin unit (EU)/mL. Autochthonous endotoxin in water samples and culture supernatants was measured after dilution according to the following scheme: tap water #1: 50- and 500-fold, tap water #2: 3- and 30-fold, deionized water #1: 5- and 50-fold, tap water #3-#6: 5- and 50-fold, culture supernatant: 5 x $10^5$- and 5 x $10^6$-fold. All water samples and the culture supernatant were tested with all amoebocyte lysate and recombinant protein reagents at three institutions: PMRJ, Japan Food Research Laboratories (JFRL) and Kyowa Kirin Co., Ltd. All water samples and the culture supernatant were subjected to testing with PyroSmart NextGen® in onset time assay and with PYROSTAR™ Neo at Musashino University. All water samples and culture supernatant were tested at participating institutions other than those mentioned above using their own amoebocyte lysate and recombinant protein reagents.

### Data analysis

*F*-test was conducted using Excel 2019 (Microsoft, Tokyo, Japan) with the software OriginPro 2022 (Lightstone, Tokyo, Japan).

## 3. Results

Water and culture supernatant samples were


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Table 1 Evaluation of Endotoxin Panel Using Amoebocyte Lysate Reagent

| No. | sample | Endochrome-K EU/mL[a] | RSD (%)[b] | 50%Min - 200%Max[c] | $n$[d] | Endospecy ES-50M EU/mL | RSD (%) | 50%Min - 200%Max | $n$ |
|---|---|---|---|---|---|---|---|---|---|
| *Autochthonous endotoxin* | | | | | | | | | |
| 1 | *Escherichia coli* O113:H10:K negative | 45880 ± 2386 | 10.40 | 20790 - 100100 | 4 | 46800 ± 2815 | 12.03 | 20030 - 107300 | 4 |
| *Water including endotoxin* | | | | | | | | | |
| 2 | Tap water #1 | 16.27 ± 1.763 | 21.67 | 5.773 - 39.93 | 4 | 15.50 ± 2.458 | 31.71 | 5.219 - 43.92 | 4 |
| 3 | Tap water #2 | 0.1845 ± 0.008788 | 9.524 | 0.07930 - 0.3940 | 4 | 0.1935 ± 0.01555 | 16.08 | 0.07930 - 0.4680 | 4 |
| 4 | Deionized water #1 | 3.692 ± 0.3117 | 16.88 | 1.473 - 8.826 | 4 | 2.926 ± 0.2375 | 16.24 | 1.156 - 6.918 | 4 |
| 5 | Tap water #3 | 3.129 ± 0.2702 | 17.27 | 1.228 - 7.555 | 4 | 2.489 ± 0.3091 | 24.84 | 0.8084 - 6.066 | 4 |
| 6 | Tap water #4 | 3.057 ± 0.2476 | 16.20 | 1.161 - 6.792 | 4 | 2.507 ± 0.07016 | 5.596 | 1.150 - 5.192 | 4 |
| 7 | Tap water #5 | 1.380 ± 0.1206 | 17.47 | 0.5832 - 3.437 | 4 | 0.9623 ± 0.05614 | 11.67 | 0.3998 - 2.079 | 4 |
| 8 | Tap water #6 | 0.7609 ± 0.06782 | 17.83 | 0.3198 - 1.761 | 4 | 0.8142 ± 0.06737 | 16.55 | 0.3365 - 1.980 | 4 |

| No. | sample | Kinetic-QCL EU/mL | RSD (%) | 50%Min - 200%Max | $n$ | PYROSTAR ES-F EU/mL | RSD (%) | 50%Min - 200%Max | $n$ |
|---|---|---|---|---|---|---|---|---|---|
| *Autochthonous endotoxin* | | | | | | | | | |
| 1 | *Escherichia coli* O113:H10:K negative | 60250 ± 4732 | 15.71 | 27000 - 148000 | 4 | 47980 ± 5148 | 21.46 | 20120 - 126300 | 4 |
| *Water including endotoxin* | | | | | | | | | |
| 2 | Tap water #1 | 14.99 ± 2.470 | 32.95 | 4.500 - 40.20 | 4 | 11.44 ± 1.224 | 21.41 | 4.358 - 28.90 | 4 |
| 3 | Tap water #2 | 0.2326 ± 0.03955 | 34.01 | 0.08055 - 0.6600 | 4 | 0.1700 ± 0.01445 | 17.00 | 0.07680 - 0.4265 | 4 |
| 4 | Deionized water #1 | 4.155 ± 0.6370 | 30.66 | 1.505 - 11.00 | 4 | 3.044 ± 0.1522 | 9.999 | 1.422 - 6.986 | 4 |
| 5 | Tap water #3 | 2.809 ± 0.4239 | 30.18 | 1.0280 - 7.980 | 4 | 3.072 ± 0.2619 | 17.05 | 1.339 - 7.600 | 4 |
| 6 | Tap water #4 | 3.644 ± 0.3677 | 20.18 | 1.386 - 8.980 | 4 | 2.920 ± 0.2633 | 18.04 | 1.170 - 6.987 | 4 |
| 7 | Tap water #5 | 1.233 ± 0.04630 | 7.513 | 0.5525 - 2.640 | 4 | 1.119 ± 0.02161 | 3.863 | 0.5360 - 2.350 | 4 |
| 8 | Tap water #6 | 1.078 ± 0.1326 | 24.61 | 0.4675 - 2.950 | 4 | 0.5857 ± 0.05223 | 17.84 | 0.2304 - 1.433 | 4 |

[a] Values shown are mean ± standard error of results from participating independent institutions.
[b] Relative standard deviation.
[c] Range between 50% of minimum and 200% of the maximum the levels detected with amoebocyte lysate reagent.
[d] Number of institutions that carried out measurements.

tested for autochthonous endotoxin with four amoebocyte lysate reagents and four recombinant protein reagents. The mean and standard error for each reagent were calculated, and the results are shown in Tables 1 and 2, and Fig. 2 and 3. Autochthonous endotoxin was detected in all water samples and the culture supernatant with both amoebocyte lysate and recombinant protein reagents. As shown by $F$-test analysis, autochthonous endotoxin levels detected with both amoebocyte lysate and recombinant protein reagents showed equal reagent-to-reagent variance among all samples, with the exception of culture supernatant (Table 3).

## 4. Discussion

In this study and the two previous studies[17, 18], the appropriateness of using recombinant protein reagents to test endotoxin as an alternative method to the BET was evaluated by ten institutions.

Autochthonous endotoxin in both culture supernatant and water samples was detected with both amoebocyte lysate and recombinant protein reagents with acceptable repeatability and reproducibility. Moreover, for each sample measured at three or more institutions, inter-institutional differences between recombinant and amoebocyte lysate reagents were similar, suggesting that there was no difference in



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Table 2 Evaluation of Endotoxin Panel Using Recombinant Protein Reagent

| No. | sample | EndoZyme II | | | PyroGene | | | PyroSmart NextGen-onset time | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | EU/mL[a] | RSD (%)[b] | *n*[c] | EU/mL | RSD (%) | *n* | EU/mL | RSD (%) | *n* |
| *Autochthonous endotoxin* | | | | | | | | | | |
| 1 | *Escherichia coli* O113:H10:K negative | 113500 ± 1189 | 2.095 | 4 | 47640 ± 5043 | 21.17 | 4 | 54700 ± 3176 | 12.99 | 5 |
| *Water including endotoxin* | | | | | | | | | | |
| 2 | Tap water #1 | 2.386 ± 0.3271 | 27.42 | 4 | 4.205 ± 0.7796 | 37.08 | 4 | 15.40 ± 1.444 | 20.97 | 5 |
| 3 | Tap water #2 | 0.02861 ± 0.0009355 | 6.539 | 4 | 0.03048 ± 0.001705 | 11.19 | 4 | 0.1890 ± 0.01822 | 21.56 | 5 |
| 4 | Deionized water #1 | 1.647 ± 0.03545 | 4.304 | 4 | 1.759 ± 0.1825 | 20.75 | 4 | 3.230 ± 0.3885 | 26.89 | 5 |
| 5 | Tap water #3 | 0.4557 ± 0.1028 | 45.11 | 4 | 0.7513 ± 0.05651 | 15.04 | 4 | 2.600 ± 0.2012 | 17.31 | 5 |
| 6 | Tap water #4 | 0.7006 ± 0.05401 | 15.42 | 4 | 0.8350 ± 0.08132 | 19.48 | 4 | 2.940 ± 0.1654 | 12.58 | 5 |
| 7 | Tap water #5 | 0.3460 ± 0.02350 | 13.59 | 4 | 0.2966 ± 0.01731 | 11.67 | 4 | 0.8130 ± 0.05953 | 16.37 | 5 |
| 8 | Tap water #6 | 0.1462 ± 0.02364 | 32.35 | 4 | 0.06563 ± 0.007883 | 24.02 | 4 | 0.7900 ± 0.05788 | 16.38 | 5 |

| No. | sample | PyroSmart NextGen-rate | | | PYROSTAR Neo | | |
|---|---|---|---|---|---|---|---|
| | | EU/mL | RSD (%) | *n* | EU/mL | RSD (%) | *n* |
| *Autochthonous endotoxin* | | | | | | | |
| 1 | *Escherichia coli* O113:H10:K negative | 50820 ± 2981 | 10.16 | 3 | 74920 ± 5946 | 17.75 | 5 |
| *Water including endotoxin* | | | | | | | |
| 2 | Tap water #1 | 14.31 ± 2.165 | 26.20 | 3 | 10.47 ± 1.774 | 37.89 | 5 |
| 3 | Tap water #2 | 0.1491 ± 0.008495 | 9.867 | 3 | 0.04996 ± 0.002622 | 11.74 | 5 |
| 4 | Deionized water #1 | 3.929 ± 0.09098 | 4.011 | 3 | 3.198 ± 0.2781 | 19.45 | 5 |
| 5 | Tap water #3 | 2.846 ± 0.3591 | 21.86 | 3 | 1.289 ± 0.1465 | 25.42 | 5 |
| 6 | Tap water #4 | 2.772 ± 0.1125 | 7.026 | 3 | 1.626 ± 0.2486 | 34.19 | 5 |
| 7 | Tap water #5 | 0.7927 ± 0.01538 | 3.360 | 3 | 0.4279 ± 0.03375 | 17.64 | 5 |
| 8 | Tap water #6 | 0.6314 ± 0.1053 | 28.87 | 3 | 0.3122 ± 0.02964 | 21.23 | 5 |

[a] Values shown are mean ± standard error of results from participating independent institutions.
[b] Relative standard deviation.
[c] Number of institutions that carried out measurements.

reproducibility.

The reagent-to-reagent variability among the four amoebocyte lysate reagents in detecting the autochthonous endotoxin level in each water sample and *E. coli* culture supernatant was 1.26-fold at minimum (Endochrome-K/Endospecy ES-50M in tap water #3) and 1.84-fold at maximum (Kinetic-QCL/PYROSTAR ES-F in tap water #6). Among the four recombinant protein reagents, the reagent-to-reagent variability was 2.38-fold at minimum (EndoZyme II/PyroGene for the culture supernatant) and 12.04-fold at maximum (PyroSmart NextGen-onset time/PyroGene for tap water #6). Some recombinant protein reagents exhibited lower detectability of autochthonous endotoxin in water samples.

In this study, we adopted the BET assay variability of 50%-200% as defined in the Pharmacopeias to investigate the comparability of endotoxin levels in samples detected by recombinant protein reagents and by the amoebocyte lysate reagents. Endotoxin levels detected by recombinant protein reagents falling within the 50%-200% range of the respective lysate reagents were considered comparable for the present purpose. PyroSmart NextGen® detected autochthonous endotoxin in all seven water samples and the culture supernatant sample at comparable levels. PyroGene™ and EndoZyme® II detected autochthonous endotoxin in two samples, deionized water #1 and the culture supernatant, at levels comparable to the lysate


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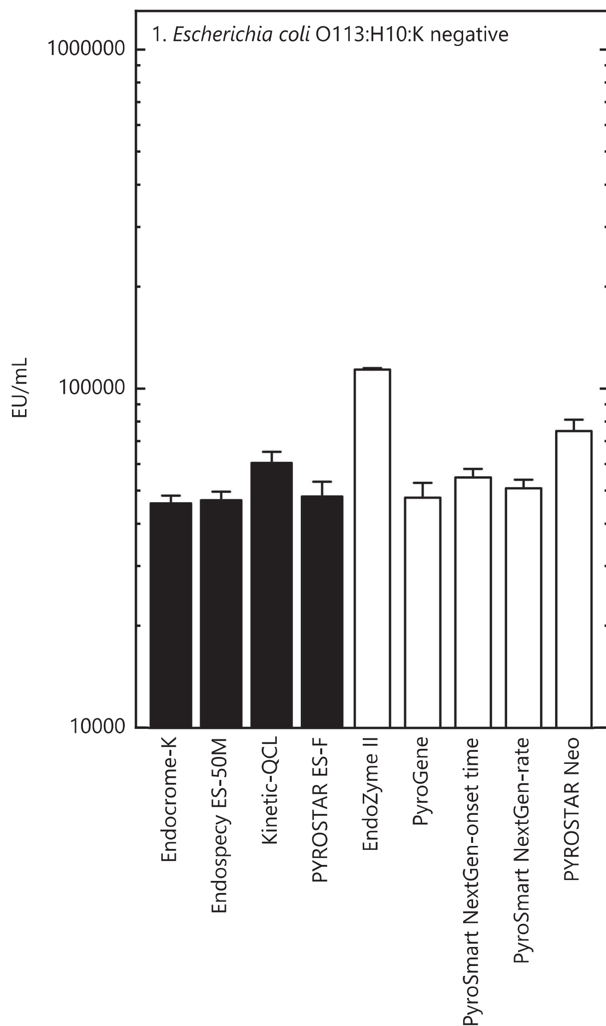


Fig. 2 Comparison of Amoebocyte Lysate and Recombinant Protein Reagents for Detection of Autochthonous Endotoxin

Culture supernatant of *Escherichia coli* O113:H10: K negative was measured with various amoebocyte lysate (■) and recombinant protein reagents (□) . The relative endotoxin activities (EU/mL) against RSE were calculated to compare the specificity of each reagent. Values are the mean ± standard error shown in Tables 1 and 2.

reagents levels. PYROSTAR™ Neo detected autochthonous endotoxin in six samples (tap water #1, 3, 4, 5 and 6, deionized water #1) and culture supernatant at comparable levels. This tendency of rFCs to detect lower levels of autochthonous endotoxin in tap and deionized water than those measured with lysate reagents was observed in our first study in 2017[17] and was previously described[7]. The reason for the difference in detectability of autochthonous endotoxin in water between rFCs and rCRs is not clear. However, one possibility is that it might be depend upon whether or not factor B is included in the recombinant protein reagents. Factor B is reported to participate in the recognition of endotoxin as well as in signal transduction within the cascade[20]. The slight differences in endotoxin detection with PyroSmart NextGen® and PYROSTAR™ Neo, which are both rCRs, might be ascribed to differences of other formulation components in those reagents. Nevertheless, the detectability of autochthonous endotoxin in the culture supernatant of *E. coli* O113: H10: K negative and most purified LPS from several types of bacteria in the previous two-year study[17, 18] by recombinant protein reagents was comparable to that with the four amoebocyte lysate reagents. The same tendency, including low measured potency of LPS such as *Helicobacter pylori* LPS, is also reported by another group[7]. These findings may suggest that autochthonous endotoxin in tap and deionized water is less able to associate with or activate factor C, compared to standard endotoxin. Further studies may be necessary to elucidate why the relative detectability of endotoxin by recombinant protein reagents and by amoebocyte lysate reagents varies depending on the origin of the endotoxin.

When selecting a commercially available recombinant protein reagent to be used for testing endotoxin, it is recommended that end-users consider aspects of product quality and development, in addition to the performance of rFC[12] or rCR[16]. Most amoebocyte lysate reagents are currently licensed by the FDA, meaning that the development and product processes comply with regulatory guidelines and current Good Manufacturing Practice (cGMP) and/or International Organization for Standardization (ISO) standards. Similar quality controls for the development, manufacturing, and master cell banking of recombinant protein reagents are critical from an end-user perspective.



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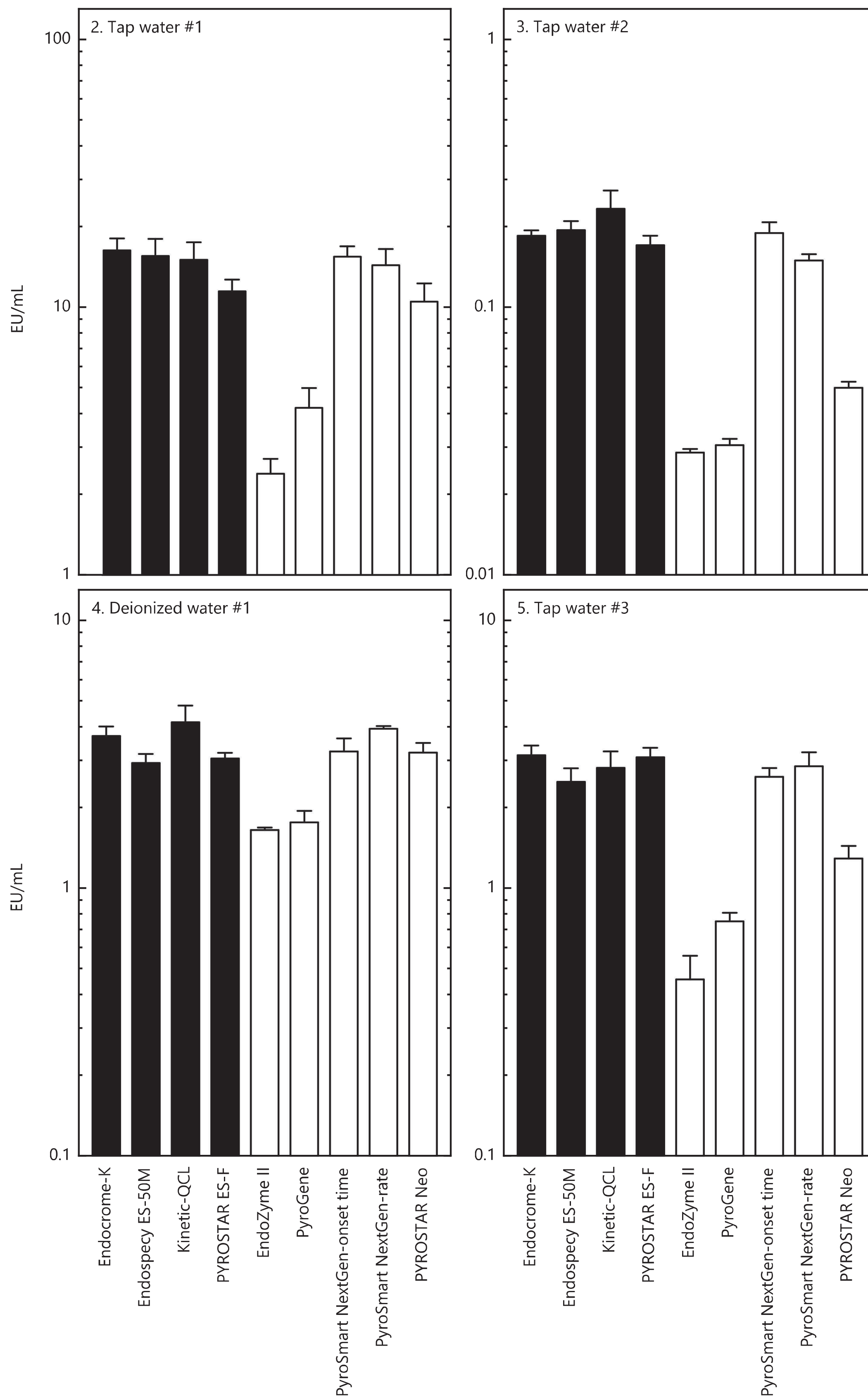


Fig. 3 Comparison of Results of Amoebocyte Lysate and Recombinant Protein Reagents for Endotoxin Panel Testing

Water samples containing endotoxin, collected from independent institutions, were tested with various amoebocyte lysate (■) and recombinant protein reagents (□) . The relative endotoxin activities (EU/mL) against RSE were calculated to compare the specificity of each reagent. Values are the mean ± standard error shown in Tables 1 and 2.


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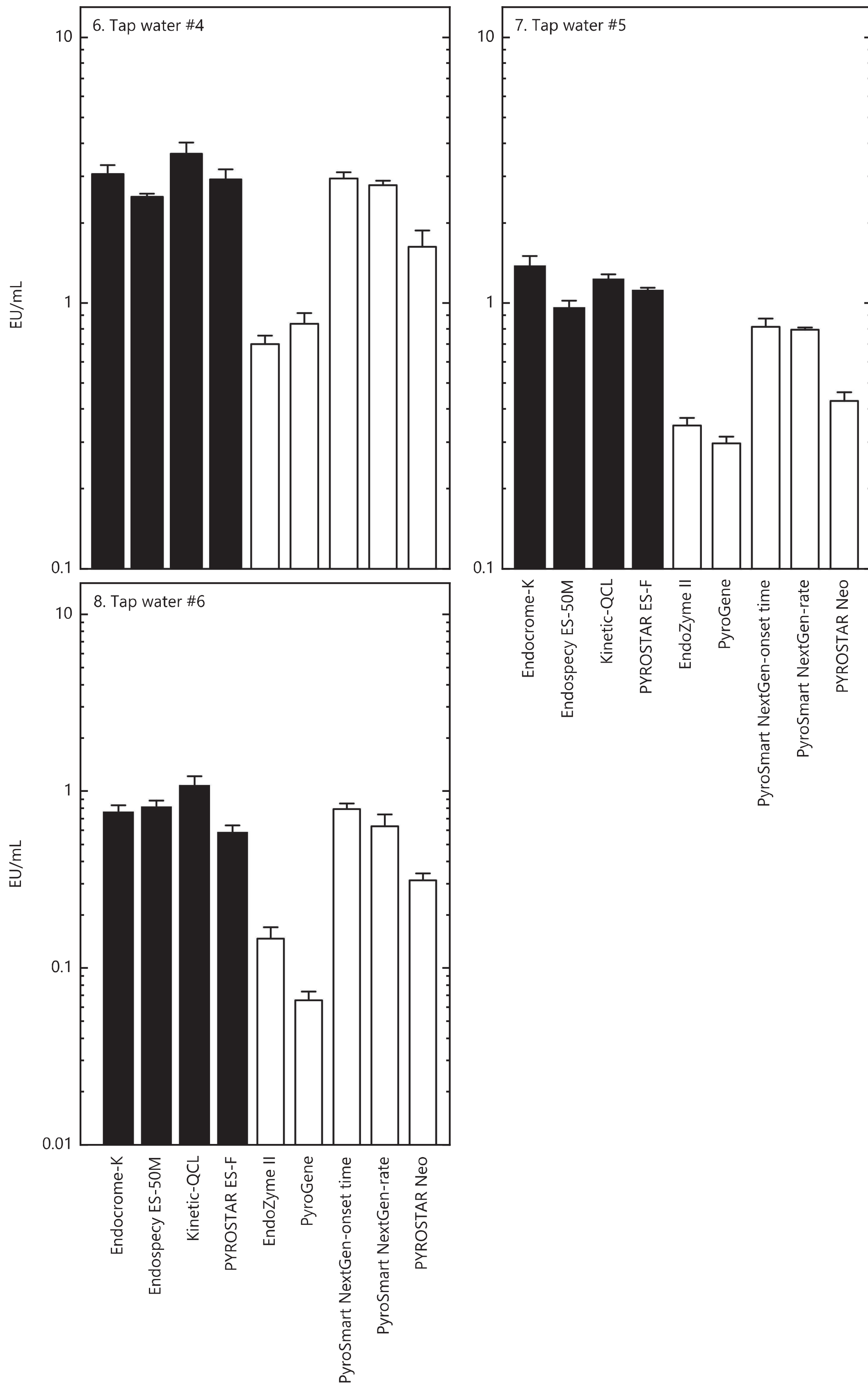


Fig. 3 Continued



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Table 3 Equality of Variacne of Endotoxin Level by Amoebocyte Lysate and Recombinant Protein Reagents

| No. | sample | *F* value[a] | critical value[b] | *P* value[c] | distribution[d] |
|---|---|---|---|---|---|
| *Autochthonous endotoxin* | | | | | |
| 1 | *Escherichia coli* O113:H10:K negative | 16.55 | 15.10 | 0.04396 | not equal variance |
| *Water including endotoxin* | | | | | |
| 2 | Tap water #1 | 7.509 | 15.10 | 0.1294 | equal valiance |
| 3 | Tap water #2 | 7.742 | 15.10 | 0.1243 | equal valiance |
| 4 | Deionized water #1 | 3.030 | 15.10 | 0.3893 | equal valiance |
| 5 | Tap water #3 | 13.67 | 15.10 | 0.05746 | equal valiance |
| 6 | Tap water #4 | 4.988 | 15.10 | 0.2178 | equal valiance |
| 7 | Tap water #5 | 1.982 | 15.10 | 0.6008 | equal valiance |
| 8 | Tap water #6 | 2.342 | 15.10 | 0.5104 | equal valiance |

[a] *F* value was calculated from the ratio of variance from the two groups
[b] *F* critical value is the value where the area is 0.05 when the degree of freedom is (4, 3) in a two-sided test in the *F* distribution table.
[c] *P* value is the probability that the null hypothesis of equal variances holds.
[d] The difference between amoebocyte lysate and recombinant protein reagent groups was considered significant when $P < 0.05$ in a two-sided test.

A further study to compare the ability of amoebocyte lysate and recombinant protein reagents to detect endotoxin from *H. pylori* is in progress.

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## Conflicts of Interest

O.M., M.S. and S.S. are employees of Hyglos/bioMérieux Japan, Ltd.; K.O. is an employee of J.K. International, Inc.; T.K., H.F. and M.K. are employees of FUJIFILM Wako Pure Chemical Corporation; T.M. is an employee of Lonza; T.O., H.M. and N.O. are employees of Seikagaku Corporation; D.I., H.S. and Y.T. are employees of Veritas Corporation; and M.T. is an employee of Charles River. The other authors declare that they have no conflict of interest.

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