Clin. Lab. 2018;64:XXX-XXX

SHORT COMMUNICATION

Comparison of the Temperature Influence on the Activity of Currently Available Procalcitonin Reagents

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SUMMARY

Background: **Procalcitonin (PCT) is a stable biomarker for bacterial infections; however, limited data is available on new trivalent reagents. We evaluated temperature influence on the activity of PCT reagents.**
Methods: **Using both conventional and trivalent reagents, we measured PCT levels of 30 clinical samples, stored residuum at refrigerator (4°C) and room temperature (24°C), and reexamined it after 24 hours. We defined a reduction rate as a percentage of PCT level at 24 hours compared to that after defrost and evaluated a ratio of reduction rate in 4°C to that in 24°C.**
Results: **The reduction rate at room temperature decreased significantly compared to that in the refrigerated condition for all the reagents examined ($p < 0.001$). In addition, the ratio of reduction rate between the conventional and trivalent reagents showed a significant difference ($p < 0.001$)**
Conclusions: **The serum PCT levels significantly decrease at room temperature, particularly when using newer trivalent reagents.**
(Clin. Lab. 2018;64:xx-xx. DOI: 10.7754/Clin.Lab.2017.170510)

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Short Communication accepted August 22, 2017

KEY WORDS

anti-N-procalcitonin antibody, bacterial infection, biomarker, refrigeration, room temperature

INTRODUCTION

Procalcitonin (PCT), a 116-amino acid precursor protein of calcitonin, is a valid biomarker that is used for early diagnosis of bacterial infections [1,2]. Compared to other inflammatory markers, PCT has greater accuracy in detecting sepsis, allowing for early initiation of treatment and, potentially, improved prognosis [3]. Also, studies have shown that PCT-guided antibiotic treatment strategies often result in proper antibiotic use [4]. In clinical laboratories, serum PCT levels are typically measured via immunoassay. PCT reagents conventionally include assays that target anti-calcitonin and anti-katacalcin: VIDAS BRAHMS PCT (VID; SYSMEX, bioMérieux Co., Ltd. Shinagawa-ku, Japan), and Elecsys® BRAHMS PCT (ECL; Roche Diagnostics Co.,

Ltd. Minato-ku, Japan). Recently, trivalent reagents that additionally target N-PCT have been launched: Chemilumi BRAHMS PCT (CEN; Siemens Healthineers Diagnostics Co., Ltd. Shinagawa-ku, Japan) and Lumipulse® BRAHMS PCT (LUM; Fujirebio Co., Ltd. Hachioji-shi, Japan) (Table 1). The new reagents are reported to have greater sensitivity than the conventional reagents; however, data regarding these reagents is limited. Our objective is to assess the *ex vivo* influence of temperature on the activity of the PCT reagents.

MATERIALS AND METHODS

Serum samples with increased PCT levels (> 5 ng/mL measured by ECL) were obtained from 30 patients and were stored at -60°C. After allowing the samples to thaw naturally, PCT levels were quantified using the conventional and trivalent reagents. After the measurements were completed, we divided each residuum into two different tubes, and subsequently stored each tube at a different temperature, one at 4°C and the other at room temperature (24°C). We then reexamined the PCT levels in each tube at 24 hours. We defined a reduction rate as a percentage of PCT level at 24 hours compared to PCT level after defrost. The reduction rates were calculated for both refrigeration and room temperature conditions in each of the four reagents. For comparison of the temperature influence on reagent activity, we evaluated a ratio of reduction rate at 4°C to that at 24°C (reduction rate at 4°C/reduction rate at 24°C) between the conventional and trivalent reagents. For statistical analysis, the Wilcoxon signed-rank test was applied using the EZR software based on R (version 3.3.1). Statistical significance was set at p-value < 0.05. Informed consent was waived given that data was collected retrospectively without the use of any personal identifiers or the application of any intervention.

RESULTS

PCT levels decreased under both the refrigerated and room temperature conditions at 24 hours. The median reduction rates (interquartile range, IQR) for VID, ECL, CEN, and LUM under refrigeration were 97.7% (96.4%, 98.5%), 96.8% (95.0%, 98.0%), 95.5% (94.3%, 96.7%), and 94.4% (91.9%, 96.2%), respectively. In contrast, those at room temperature decreased to 87.4% (80.7%, 93.3%), 85.9% (78.0%, 93.6%), 78.5% (72.1%, 85.4%), and 78.6% (72.4%, 84.4%), respectively. The reduction between defrost and at 24 hours showed linear changes in both conventional and trivalent reagents by examining PCT levels at 12 hours (data not shown). The reduction rates between the refrigerated and room temperature conditions were significantly different for all the reagents examined ($p < 0.001$) (Figure 1). The reduction rate observed in the trivalent reagents were significantly lower than that in conventional reagents under both temperature conditions ($p < 0.001$). Finally, the ratio of the reduction rate between the conventional and trivalent reagents showed a significant difference ($p < 0.001$), indicating that the reduction rates of the trivalent reagents were larger than those measured by conventional ones at room temperature.

DISCUSSION

The present study revealed that PCT levels after 24 hours were significantly decreased when stored at room temperature compared to refrigeration. According to a previous survey, the reduction rates of PCT levels under refrigerated and room temperature conditions after 24 hours were 6.3% and 12.4%, respectively [5]. In our study, the median reduction rates for VID, ECL, CEN, and LUM under refrigeration were 2.3%, 3.2%, 4.5%, and 5.6%, respectively, which were similar to previously reported values. In contrast, under the room temperature condition, the median reduction rates for VID and ECL were 12.6% and 14.1%, while those of CEN and LUM significantly decreased to 21.5% and 21.4%.

PCT is composed of three components: N-PCT at the N-terminal side, calcitonin in the middle, and katacalcin at the C-terminal side. Anti-calcitonin and anti-katacalcin antibodies are applied as either the solid-phase or labeled-phase in the conventional reagents. In the newer reagents, the anti-N-PCT antibody is added to increase sensitivity as shown in Table 1. We believe that the differences observed in the influence of temperature between the conventional and trivalent reagents can be attributed to an enzymatic cleavage between N-PCT and calcitonin. Another possibility is a structural change of N-PCT that may be promoted at room temperature. As compared to other inflammatory markers such as cytokines, PCT is highly stable at various storage conditions [5]. However, our results showed that trivalent reagents may particularly fail to accurately measure PCT levels in blood samples that were stored at room temperature. It is recommended that PCT measurements be performed immediately after blood sampling, and, hence, we measure the PCT values shortly after samples arrive in routine work. However, some medical facilities are required to outsource PCT level measurement to external laboratory centers with greater lab capabilities. In such cases, it is important that blood samples are refrigerated and not stored at room temperature after serum separation in order to measure accurate PCT values. In addition to differences between the divalent and trivalent reagents, variation may also exist among the four individual reagents with regard to temperature effect. Thus, further investigation is warranted.

Table 1. Differences of procalcitonin (PCT) quantitative reagents.

		Conventional reagents		Trivalent reagents	
		VID	ECL	CEN	LUM
Antibody	Solid-phase	Anti-CT	Anti-KT	Anti-CT/Anti-KT	Anti-N-PCT/Anti-CT
	Labeled-phase	Anti-KT	Anti-CT	Anti-N-PCT	Anti-KT

VID - VIDAS BRAHMS PCT [SYSMEX, bioMérieux Co., Ltd.], ECL - Elecsys® BRAHMS PCT [Roche Diagnostics Co., Ltd.], CEN - Chemilumi BRAHMS PCT [Siemens Healthineers Diagnostics Co., Ltd.], and LUM - Lumipulse® BRAHMS PCT [Fujirebio Co., Ltd.].
CT - calcitonin, KT - katacalcin.

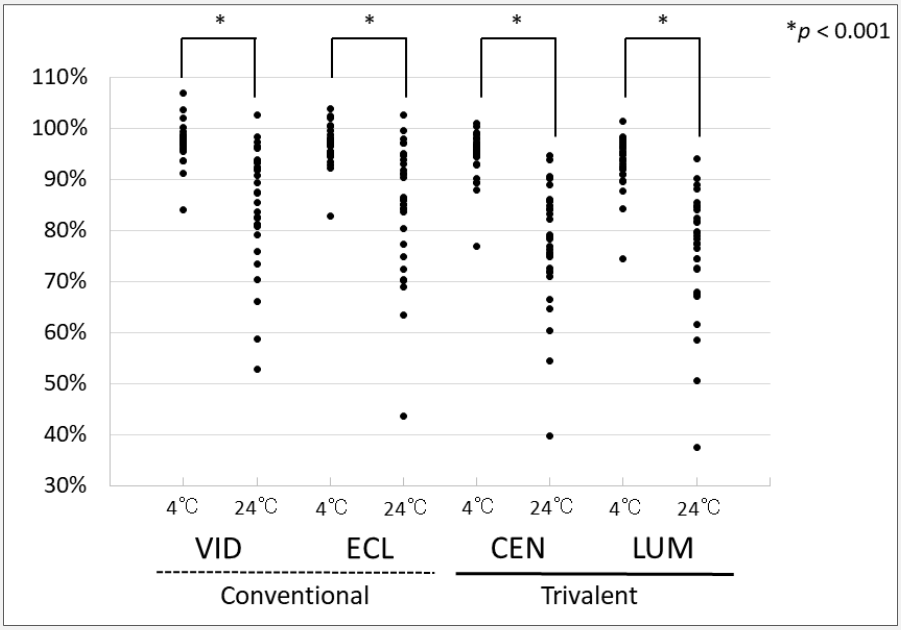

Figure 1. Reduction rates in refrigeration and room temperature conditions among the four procalcitonin reagents.

VID - VIDAS BRAHMS PCT [SYSMEX, bioMérieux Co., Ltd.], ECL - Elecsys® BRAHMS PCT [Roche Diagnostics Co., Ltd.], CEN - Chemilumi BRAHMS PCT [Siemens Healthineers Diagnostics Co., Ltd.], and LUM - Lumipulse® BRAHMS PCT [Fujirebio Co., Ltd.]. * - p < 0.001.

CONCLUSION

In summary, our study showed that serum PCT levels were significantly reduced when stored at room temperature, a finding that was more pronounced among trivalent reagents.

Authors' Contributions:
Study conception and acquisition of data: M. Deguchi and N. Yoshioka. Drafting of manuscript: H. Hagiya and N. Yoshioka. Statistical analysis: H. Hagiya, N. Yoshioka, and S. Yukawa. Critical revision: Y. Hidaka and K. Tomono.

Funding:
None to report.

Declaration of Interest:
The authors declare that they have no competing interests.

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