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RESEARCH ARTICLE

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Analytical and clinical evaluation of a novel assay for measurement of interleukin 6 in human whole blood samples

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Abstract

Background: Interleukin 6 assays are useful in early detection of infections and risk stratification of critically ill patients, so an assay with a short turnaround-time and near-patient use is preferred. This study evaluated the performance of a new interleukin 6 assay, Pylon IL-6 assay, and explored its potential use in near-patient settings.

Methods: We carried out imprecision, linearity and comparison studies using serum and plasma samples according to CLSI EP guidelines. The stability of whole blood samples during storage was assessed. Furthermore, whole blood samples from pediatric patients with suspected infection were measured to evaluate the assay's diagnostic performance.

Results: The within-run CVs and total CVs of Pylon IL-6 assay were determined as 1.8% and 3.0% at 159.3 pg/ml and 3.5% and 4.7% at 8009.9 pg/ml, respectively. The method showed linearity between 1.5 and 42,854 pg/ml. The results of serum samples measured by Pylon assays correlated to those measured by Roche assays, as well as to those of matched whole blood samples measured by Pylon assays. IL-6 in whole blood was found stable for ~8 h at room temperature. Pylon IL-6 results of whole blood samples from 179 pediatric patients with suspected infection showed an AUC of 0.842 in diagnosis of bacterial infection. The turnaround time of Pylon IL-6 assay was only 1 h when using whole blood samples.

Conclusion: The new assay demonstrated performance comparable to those performed on clinical laboratory instruments and can be used in near-patient settings with whole blood to reduce turnaround times.

KEYWORDS
immunoassay, Interleukin 6, near patient, pediatrics, procalcitonin, whole blood

Zhicheng Ye and Niuniu Dong contributed equally to this work.

1 | INTRODUCTION

Interleukin 6 (IL-6) is a pleiotropic cytokine produced by various stromal cells and immune system cells, involved in inflammation and infection responses, regeneration process, metabolism regulation, bone homeostasis, and neural functions, etc.[1] It is indicated in many diseases such as infection, injury, trauma, stress, neoplasia, post-surgery complications, and chronic inflammatory diseases.[2]

In outpatient departments and emergency department (ED), IL-6 is useful for screening bacterial infection and sepsis in febrile patients and predicting later complications in trauma patients.[3,4] In intensive care units (ICU), IL-6 levels are useful in evaluating severity of systemic inflammatory response syndrome and sepsis and predicting outcomes of patients.[5,6] In the current COVID-19 pandemic, a high level of IL-6 at the admission predicts ICU placement and poor outcomes.[7] A short turnaround time (TAT) from sample collection to results report of IL-6 assays can facilitate patient management in those clinical settings. However, most IL-6 assays are only available on large laboratory-based instruments using plasma or serum samples with prolonged TATs.[8]

Here, we evaluated a new Pylon IL-6 assay performed on a benchtop immuno-analyzer that can use whole blood (WB) samples, and explored its usability in near-patient settings to reduce TATs for improvement in patient care.

2 | MATERIALS AND METHODS

2.1 | IL-6 measurements

The study evaluated the performance of the Pylon IL-6 assay (ET Healthcare, China) based on a cyclic enhanced fluorescent immunoassay performed on a fully automated, benchtop Pylon 3D immunoanalyzer. A description of assay procedures is available elsewhere.[9] In brief, the assay format is a unitized test strip containing wells with pre-dispensed reagents. A quartz-glass probe tip coated with capture antibodies moves between wells to capture sample IL-6 and form immune complexes with detection antibodies, before fluorescent polymers are bounded to the immune complexes on the tip through biotin and streptavidin interaction. After measuring fluorescence bound on the probe tip, the IL-6 concentration is derived from a reagent lot specific calibration curve.

The whole assay process is completed by Pylon 3D immune-analyzer automatically in about 10 min after patients' blood collection tubes and Pylon test strips are loaded in the analyzer. When measuring WB samples, the analyzer's pipetting subsystem senses hematocrit at each sampling from individual collection tubes and a software algorithm corrects for hematocrit in WB before the WB results are reported.

2.2 | Precision study

Two pooled serum samples supplemented with IL-6 (~150 and ~8000 pg/ml) were aliquoted and stored at −80°C. For five consecutive days, an aliquot from each sample was thawed and measured in triplicate. Within-run and total analytical imprecision was calculated according to CLSI EP 15-A2.

2.3 | Linearity study

A low-concentration pool was prepared using patients' samples close to limit of detection. A high-concentration pool was prepared by spiking recombinant IL-6 (Sigma Aldrich, product no. I1395) into an aliquot of the pooled sample to a final concentration of ~45,000 pg/ml. Six different analyte concentrations were prepared by directly mixing the low-concentration and high-concentration sample pool in the ratios of 5:0, 4:1, 3:2, 2:3, 1:4, and 0:5. Samples of each concentration was measured in duplicates. Similarly, linearity was also evaluated using five patients' serum samples of various concentrations. The results were analyzed according to CLSI EP 6A.

2.4 | Comparison study

A total of 100 remnant serum samples from outpatients and ED patients were measured by the Pylon assay and the Elecsys IL-6 assay on a Cobas e 602 (Roche). To compare the results of different sample types, EDTA-anticoagulated WB samples were tested against matched serum samples using the Pylon assay. All the samples were stored at 4°C and measured within 8 h of collection. Passing-Bablok regressions were used for analysis.

2.5 | Stability study

Ten WB samples from patients (concentration range, 50–3000 pg/ml) were divided into 2 vials each and stored at room temperature and 4°C respectively up to 72 h. The initial concentrations of the samples were determined and the samples were measured after 1, 2, 4, 6, 8, 12, 24, 48, and 72 h of storage. Relative analyte recovery of samples was calculated as the percent of the baseline value for each sample at each given time interval of storage.

2.6 | Clinical evaluation study

Pediatric patients, admitted to the ED department of the Children's Hospital of Fudan University between April and September 2020 with suspected infection, were included in the study. Patients went through routine diagnostic workup according to hospital standard procedures. Blood samples and respiratory tract samples were collected for routine clinical laboratory tests, that is, hematology tests, CRP, PCT, viral antigen, or antibody tests and microbiology culture tests. The positive bacterial culture results of blood, urine, or sputum suggest a bacterial infection. While the viral infection was diagnosed according to the following criteria: positive results of the viral antigens

by antigen-based tests and/or a positive IgM antibody through serological assays and also a negative bacteriological result. Negative results of microbial culture and virology tests, combined with normal imaging examination and clinical symptoms, the patients were assigned to a non-infected group. Remnant EDTA-anticoagulated WB samples were used for measuring IL-6 levels by the Pylon assay. ROC curves were constructed to assess the diagnostic accuracy of biomarkers for infection. Study procedures were in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Children's Hospital of Fudan University (no. [2018]275).

2.7 | Statistical analysis

Statistical analyses were performed with MedCalc. All hypothesis testing was two-tailed and p values of <0.05 were considered to indicate statistical significance.

3 | RESULTS

3.1 | Precision

The Pylon IL-6 assay had a within-run CV of 1.8% and a total CV of 3.0% at a mean concentration of 159.3 pg/ml (pool 1) and a within-run CV of 3.5% and a total CV of 4.7% at a mean concentration of 8009.9 pg/ml (pool 2).

3.2 | Linearity

The low-concentration pool had a mean IL-6 level of 1.5 pg/ml, while the high concentration pool had a mean of 42,854 pg/ml. The standard errors of regression (S_{yx}) and t test from regression analyses showed that the first-order model fitted best: first-order model b_1, $t = 38.183$ ($p < 0.0001$); second-order model b_2, $t = 0.863$ ($p = 0.795$); third-order model b_3, $t = 0.532$ ($p = 0.331$) and b_2, $t = 0.434$ ($p = 0.348$) (Figure 1). Similarly, the methods showed linear when evaluating with five clinical samples between 4336 and 40,893 pg/ml (Figure S1–S5 and Table S1–S5 in Supplementary data). Thus, the method was linear within 1.5–42,854 pg/ml.

3.3 | Method comparison and sample type comparison

The results of 100 serum samples measured by the Pylon assay and the Elecsys assay showed correlation (Spearman's rho = 0.997, $p < 0.0001$) and Passing-Bablok analysis indicated a linear regression (slope = 1.084). (Figure 2a).

IL-6 concentrations measured in 100 WB and matched serum samples by the Pylon assay showed correlation (Spearman's rho = 0.995, $p < 0.0001$) and Passing-Bablok analysis indicated a linear regression (slope = 0.932) (Figure 2b).

FIGURE 1 Linearity study using recombinant IL-6 showed to best fit with first-order model (1.5–42,854 pg/ml)

3.4 | Stability study

The results of the stability study were shown with IL-6 values displayed as relative analyte recovery at various time points between 0–72 h (Figure 3). It was clear to see that the overall recovery rates dropped much faster at room temperature. Thus, samples were more stable by storage at 4°C. Applying the criteria for stability as 90%, which was about 2–3 times of assay CVs, IL-6 in WB was stable for about 8 h at room temperature and for about 48 h at 4°C. At both time points of respective storage condition, fewer than 20% WB samples had recovery rates of less than 90%.

3.5 | Clinical evaluation

The samples of a total of 179 pediatric patients (107 males, 59.8%) in the ED were included. The median age of these patients was 3 years old (IQR 1–8). Eighty-four patients were diagnosed with bacterial infections including one tuberculosis infection and one mycoplasma infection, and the other 95 patients included 27 viral infections, 2 co-infections, and 66 non-infections. The details of infections were provided in Table S6 in Supplementary Data. The AUC for diagnosis of infection (113 infections vs. 66 non-infections) was 0.865 (95% CI 0.806–0.912, $p < 0.0001$); and that for diagnosis of bacterial infection (86 bacterial infections and co-infections vs. 93 non-bacterial infections and virus infections) was 0.842 (95% CI 0.780–0.892, $p < 0.0001$) (Figure 4), comparable to the AUC of PCT for bacterial infection (AUC = 0.912; 95% CI, 0.861–0.949).

It is noticed that IL-6, CRP, and PCT levels of the group of bacterial infections showed more pronounced increased than the group of

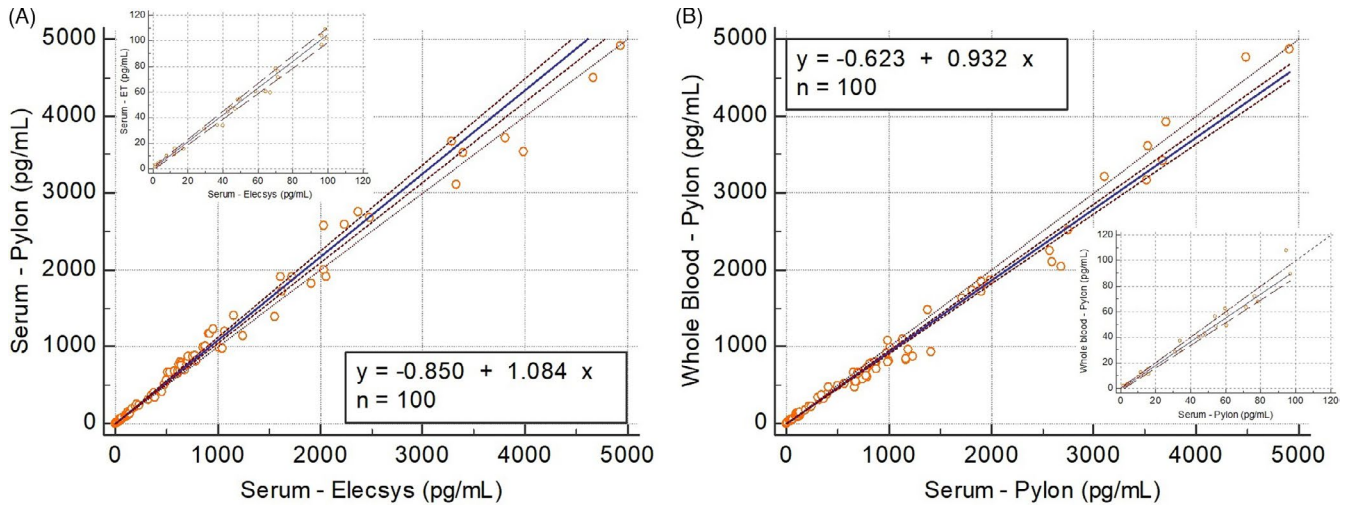

FIGURE 2 Correlations (Passing-Bablok) between the Pylon and Elecsys IL-6 assay (A) and between Pylon serum and WB results (B). Inserts are the correlation of samples lower than 100 pg/ml

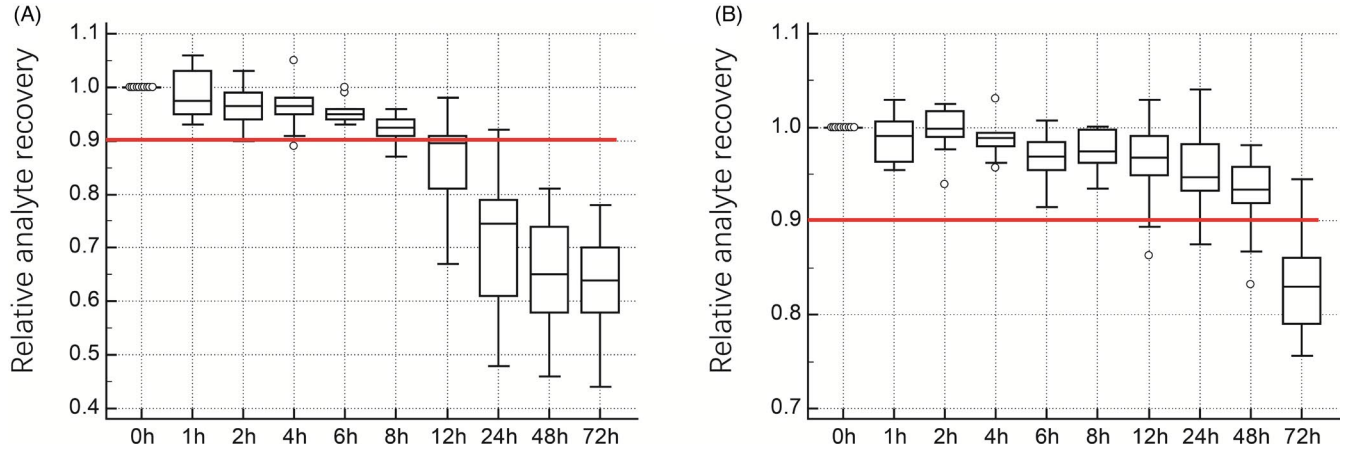

FIGURE 3 Stability study of IL-6 in 10 WB samples at room temperature (A) and 4°C (B). Recovery rates were calculated as percentages of baseline concentrations. Box-and-whisker plots showed the distribution of recovery rates across 10 samples at each time points. The red dash line is the recovery rate of 90%

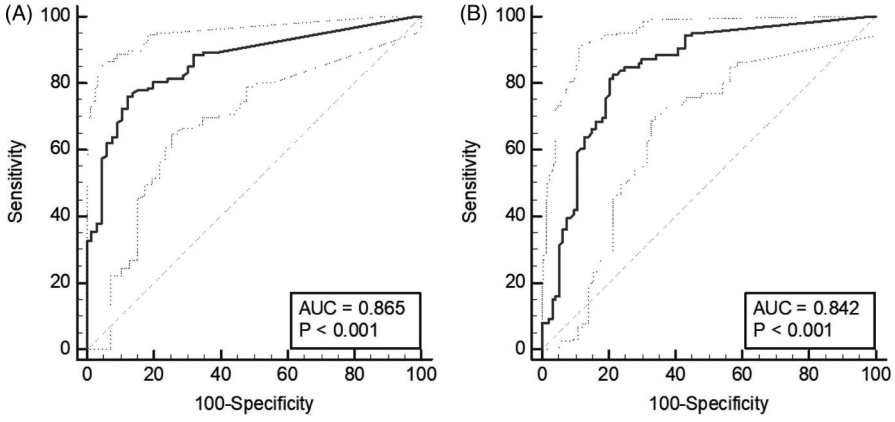

FIGURE 4 ROC analysis of IL-6 for diagnosis of infection (A) and bacterial infection (B)

virus infections (Table 1). Moderate positive correlations were found between IL-6 and PCT (Spearman's rho = 0.545; $p < 0.001$) and between IL-6 and CRP (Spearman rho = 0.585; $p < 0.001$) in all patient samples.

4 | DISCUSSION

IL-6 is known for its use in assessing patients with suspected infection or sepsis.[3] During the outbreak of the COVID-19, its use in risk

TABLE 1 Levels of inflammatory biomarkers in non-infection, virus infection, and bacterial infection groups

	Non-infection ($n = 66$)	Virus infection[a] ($n = 27$)	Bacterial infection[a] ($n = 84$)	p
Age, median (IQR[b]), years	5.5 (2.0 to 9.0)	3 (1.0 to 4.8)	2 (0.96 to 6.5)	0.001181[c]
Gender, (n, %)	Male 34, 51.5%	Male 16, 59.3%	Male 56, 66.7%	0.1706[d]
IL−6, median (IQR), pg/ml	1.5 (1.5 to 4.0)	8.2 (1.5 to 33.2)	30.0 (14.9 to 73.7)	<0.0001[c]
PCT, median (IQR), ng/ml	0.04 (0.04 to 0.05)	0.07 (0.04 to 0.13)	0.965 (0.20 to 2.50)	<0.0001[c]
CRP, median (IQR), mg/L	8 (8.0 to 8.0)	8 (8.0 to 8.0)	46.1 (18.4 to 97.0)	<0.0001[c]
WBC, median (IQR), $\times 10^9$/L	7.8 (5.9 to 10.7)	6.3 (5.5 to 9.5)	10.2 (6.9 to 15.5)	0.002341[c]

[a] Co-infections were not included.
[b] IQR: Interquartile Range.
[c] comparison between groups using Kruskal-Wallis test.
[d] comparison between groups using Chi-squared test.

stratification of ED and ICU patients is more relevant.[10] Thus, an IL-6 assay with a short TAT is preferred especially in ED, ICU, and outpatient departments, while most IL-6 assays performed in the clinical laboratories had prolonged TATs.[8] In this study, we evaluated a new Pylon IL-6 assay for the potential to reduce TAT and use in near-patient settings.

The within-run CVs and total CVs of the assay were less than 5% in the imprecision study. The assay showed linearity over a wide concentration range (1.5–42,854 pg/ml), which is much wider than the detection range of the Elecsys IL-6 assay (1.5–5000 pg/ml). Comparison of the Pylon assay with the Elecsys assay using serum samples demonstrated good correlation over a wide concentration range (up to 5000 pg/ml) with concordance at low levels (<100 pg/ml). Thus, the Pylon IL-6 assay demonstrated comparable analytical performances to the assay performed on a laboratory-based instrument.

Furthermore, we compared the results of WB and serum samples. Concordance of the two sample types was demonstrated. The stability study showed WB samples can be considered stable for about 8 h at room temperature or 48 h at 4°C. In the clinical evaluation using WB samples of the ED pediatric patients, IL-6 increased in the patients with infections while bacterial infections led to more pronounced increase of IL-6 than viral infections. The AUCs for the detection of infections and bacterial infections were 0.865 and 0.842, respectively ($p < 0.001$). These results confirmed the accuracy of measurement in WB and the diagnostic value of WB results in infection, and thus supported the clinical use of WB samples for measuring IL-6.

Finally, the data from our Laboratory Information System showed the median and the 90th percentile TAT for the Pylon assays were 43 and 57 min, respectively, while those for the ECLIAs were 609 and 1534 min, respectively. Thus, with the implementation of Pylon IL-6 assay using WB, TAT of the IL-6 tests in the ED and the outpatient department decreased to only 1 h, compared to over 24 h on a big laboratory-based instrument for the inpatient of our hospital. The smaller size and automation of Pylon 3D analyzer and the use of WB samples with Pylon IL-6 assays simplify the workflow and allows the use in the near-patient settings. In our hospital, IL-6 is regularly ordered by departments of Hematology, Dermatology, and Rheumatology for monitoring the onset of inflammation and cytokine storm, evaluating the treatment of atopic dermatitis and urticaria and adjusting the doses of therapeutic anti-IL-6 receptor antibody. With a short TAT, it is efficient to triage outpatients and provide risk stratifications to in hospital patients for better medical care and outcome.

In conclusion, the new Pylon IL-6 assay demonstrated comparable performance to those assays performed on clinical laboratory instruments. Further, the feature of WB sampling offers advantages in ED or other near-patient settings where TAT is critical.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: Jin Xu, Zhicheng Ye, Niuniu Dong. Formal analysis: Zhicheng Ye, Niuniu Dong, Keqing Liang, Haixia Hu. Investigation: Zhicheng Ye, Niuniu Dong, Keqing Liang, Haixia Hu. Methodology: Zhicheng Ye, Niuniu Dong, Keqing Liang, Haixia Hu. Writing - original draft: Zhicheng Ye, Niuniu Dong. Writing – review & editing: Jin Xu, Zhicheng Ye, Niuniu Dong, Keqing Liang, Haixia Hu.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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