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Research article

Application of random quality control based on time -series model in ELISA detection of blood donors in Nanjing

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ABSTRACT

This study introduces a completely Random Quality Control (R-QC) method for Enzyme-Linked Immunosorbent Assay (ELISA) tests and compares it with traditional Fixed Quality Control (F-QC) methods across different testing items. A random quality control system was constructed using a Time-series model, enabling fully random positioning of quality control in ELISA tests. The Coefficient of Variation (CV) for R-QC were found to be lower as compared to F-QC in Hepatitis B surface antigen (HBsAg), anti-Human Immunodeficiency Virus (anti-HIV), and Treponema Pallidum (TP) items, and there were significant differences among them. Moreover, the alarm rates and out of control rates in the R-QC groups were notably lower than those in the F-QC groups. The experimentally designed R-QC method demonstrates superior stability, reducing alarm and out of control rates, with has significant implications for improving ELISA testing at the Nanjing Blood Center.

1. Introduction

With the continuous advancement of medical technology, the demand for blood in clinical settings has been increasing annually. Since blood is an irreplaceable resource that cannot be synthesized artificially or substituted by medications, it must be acquired through donations to meet clinical requirements [1,2]. Thus, ensuring the safety of blood products stands as the primary responsibility of blood collection and supply institutions [3,4]. Enzyme-linked Immunosorbent Assay (ELISA) plays a crucial role in blood screening at these institutions. Throughout the testing process, the efficacy of experiments can only be determined through the reagent's negative and positive controls. To enhance the precision of blood testing, it is essential to utilize quality control products to monitor experimental accuracy and reliability [5–10].

Quality control products encompass Fixed Quality Control(F-QC) and Random Quality Control(R-QC). F-QC limited in clinical applicability, cannot fully represent actual specimens or handle unexpected scenarios such as instrument or reagent failures [11]. In ELISA testing, its shortcomings primarily manifest in its inability to monitor every well on the ELISA plate and detect edge effects on detection results [12,13]. Conversely, R-QC helps identify potential errors in instruments or reagents during testing, such as missing samples or inadequate testing volumes, thereby reducing the need for retesting and optimizing resource allocation [14,15]. Moreover, R-QC reduces the likelihood of false positive or false negative results, thereby enhancing the reliability of ELISA diagnoses [16].

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Overall, R-QC serves as a critical method to enable laboratories to better support clinical diagnosis and treatment.

Time-series models have advantages in real-time updates, periodicity, and repeatability without human intervention [17]. With the development of artificial intelligence, these models have been widely applied in quality control [18–20]. For example: Real-time quality control plans can detect random errors [21]; Real-time quality control methods can detect whether the data is uniformly distributed [22]; Real-time quality control methods based on random forest algorithms can detect small shifts in the hs-cTnT project [23]; Additionally, Python programs have been developed for ELISA quality control [24]. These new quality control methods are increasingly favored by laboratories as supplements to traditional fixed approaches [25,26]. The current R-QC scheme involves randomly placing quality control products in samples for testing. However, this method disrupts sample continuity and is susceptible to human error [12]. If there are many items, it will easily to cause sample addition errors and even pollution, which will affect the reliability of the test results. How to realize automatic random sampling of quality control products in ELISA testing has not yet been reported. The R-QC method developed in our laboratory integrates seamlessly with the sample addition system, automating the addition of quality control products and ensuring their complete randomization on the ELISA plate. We collected blood samples from donors from July to September 2022, testing for Hepatitis B surface antigen (HBsAg), anti-Human Immunodeficiency Virus (anti-HIV), and Treponema Pallidum (TP) to assess the feasibility of our R-QC method. Subsequent sections will elaborate on the findings of this study.

2. Materials and methods

2.1. Layout

In the ELISA plate, there are 12 columns. The first column is designated for the Negative Control (NC), Positive Control (PC), Quality Control (QC), and Negative Quality Control (N-QC). The remaining 11 columns are allocated for the addition of specimens, coded from 1 to 88, arranged from top to bottom and left to right (Fig. 1).

2.2. Establishment of mathematical model

Random numbers following a uniform distribution within the range of 0–1 were generated in accordance with the Monte Carlo algorithm. The mathematical model for the R-QC position is given by: $a_{random\ number}$*s. '$a_{random\ number}$' is any random number between 0 and 1, and 's' signifies the number of test specimens in batches, ranging from 1 to 88.

2.3. Programmatic calculation of R-QC position

By adopting the editor of automatic sampling system, the last digit of current experiment time "Minute" and "Seconds" were converted into ASCII code as N1 (example: 2022-09-01 17: 15: 18, N1 = 58). Invoked random number generator of the MthR01Draw() function to generate arandom number (example: arandom number = 0.26), and obtained N2 according to the mathematical model arandom number*s (example: s = 43, N2 = 0.26*43 = 11.18 ≈ 11). In order to better simulate the real random situation, further expand the random range and uncontrollability, and ensure that the randomness of the result is more in line with the actual needs and expectations, N2 was repeated N1 times to be N3 (example: repeat N1 = 58 times, N3 = 40), which is the position of R-QC (Fig. 2). In extreme cases, if the value taken happens to be 0, it means that the quality control position will remain unchanged in the original hole. According to the above method, the R-QC was placed on the ELISA plate randomly.

2.4. Specimen source

According to the standard operating procedures, 5 mL of venous blood were collected from blood donors, and the supernatant was taken after centrifugation at 1500*g* for 15 min. This study received approval from the Ethics Committee, and informed consent was obtained from all blood donors.

A		1	9	17	25	33	41	49	57	65	73	81
B	NC	2	10	18	26	34	42	50	58	66	74	82
C	NC	3	11	19	27	35	43	51	59	67	75	83
D	PC	4	12	20	28	36	44	52	60	68	76	84
E	PC	5	13	21	29	37	45	53	61	69	77	85
F	QC	6	14	22	30	38	46	54	62	70	78	86
G	N–QC	7	15	23	31	39	47	55	63	71	79	87
H		8	16	24	32	40	48	56	64	72	80	88

Fig. 1. Sample position on ELISA plate.

Fig. 2. The changed position of R-QC with time 17:15:18 and 43 specimens.

2.5. Reagent and apparatus

Quality control products: Lot No. 202207009, supplied by Beijing Conchestan Biotechnology Co, Ltd, Beijing, China; ELISA detection kits: Lot No. 202212361, 202209261, 202210122, provided by InTec Products, INC, Xiamen, China; Apparatus: The Starvenas automatic sampling system (STAR-VENAS) provided by Hameidon, Switzerland.

2.6. Quality control judgment

Small data fluctuation, lower dispersion, and well-controlled situation are important indicators for judging the quality control process. In this study, we focus on the stability of the mean value, coefficient of variation, alarm rate, and out of control rate as key parameters to compare R-QC and F-QC. The ELISA was performed strictly according to the instructions provided with the reagents, and the quality control product was tested concurrently with routine specimens. Alarm is indicated if a quality control reading exceeds ± 2SD (standard deviations) but is less than ± 3SD. Out of control is indicated if a quality control reading exceeds ± 3SD or if there are 10 consecutive quality control readings on the same side of the mean.

2.7. Statistic analysis

To minimize the impact on mean and standard deviation and improve data quality, all normally distributed data from the HBV (F-QC, R-QC), HIV (F-QC, R-QC), and TP (F-QC, R-QC) groups in this experiment were subjected to outlier exclusion using the American Clinical and Laboratory Standards Institute (CLSI) C28.A2 D/R < 1/3 rule: D = maximum (small) value of all data - adjacent maximum (small) value, R = maximum value of all data - minimum value of all data [27]. The measurement data were reported as Mean ± Standard Deviation ($\bar{X}$ ± SD). One-way analysis of variance (ANOVA)was employed to compare mean differences among multiple independent groups of HBV (F-QC, R-QC), HIV (F-QC, R-QC), and TP (F-QC, R-QC). Data analysis was conducted using SPSS software, version 22.0. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Position and frequency of quality control products

In this study, a comprehensive dataset consisting of results from 300 batches (with each batch including a full ELISA plate of 88 specimens) was meticulously compiled. This rigorous data collection ensured comprehensive coverage of all 88 wells on the ELISA plate, leaving no position untested. The locations and frequencies of the R-QC system were systematically recorded and depicted (Figs. 3 and 4). Given the modest size of the test batches and the variations observed in the frequency across different wells, the study made use of Microsoft Excel's RAND(.) function to simulate 60,000 iterations [28], enabling a more detailed analysis of the

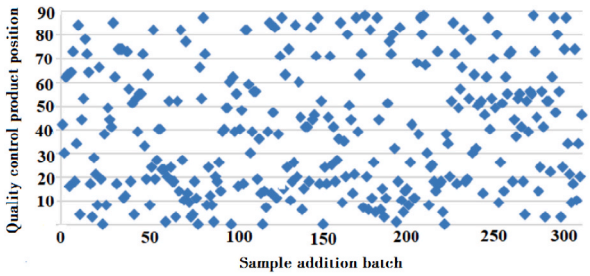

Fig. 3. Position of quality control product.

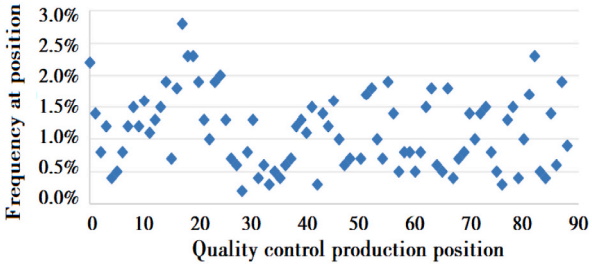

Fig. 4. Frequency of quality control product at position.

experimental results. The post-simulation frequency distribution of the R-QC positions was shown (Fig.5). The findings suggest that the frequency distribution across each well position tends to even out with the increasing size of the batches, indicating more uniform coverage.

3.2. Statistical analysis results

A statistical analysis was conducted on a total of 300 batches. Following the application of the $D/R < 1/3$ rule as defined by CLSI, no extreme values were detected, and the R-QC values demonstrated a normal distribution across all groups. The experimental design included six distinct groups: HBsAg (F-QC), HBsAg (R-QC), anti-HIV (F-QC), anti-HIV (R-QC), TP (F-QC), and TP (R-QC). The analysis revealed that the mean values of the F-QC groups were significantly higher than those of the R-QC groups. Conversely, CV of the R-QC groups was significantly lower than that of the F-QC groups (Table. 1). These differences were found to be statistically significant, with $P < 0.01$. Furthermore, the rates of alarm and out of control in the R-QC groups were markedly lower compared to the F-QC groups.

4. Discussion

The ELISA plate is a poor thermal conductor, creating a thermodynamic gradient between the peripheral and intermediate holes, which might bring edge effects during regular testing [12,13]. Conventionally, F-QC is positioned in the first column of the ELISA, but this way fails to detect missed samples due to factors like uneven heating in the incubator, loose door closure, absent samples, or insufficient test reagents [29]. Nevertheless, the randomized quality control automatic sampling program developed in this study can effectively identify these problems, avoiding false negative results and greatly enhancing the accuracy and safety of blood testing. Additionally, this approach simplifies operational procedures, reduces human error, and brings significant enhancements to the routine detection processes in our laboratory.

The quality control protocol employed in this study hinges on a time-variable mathematical model, ensuring that each initiation of a quality control instance by the automated system is distinct. The uniqueness of each activation, determined by the time of launch, fosters a high degree of randomness in specimen selection, leading to unpredictable yet representative outcomes. Our data imply that when the number of tests per batch is ample, the distribution of R-QC across different well positions tends to be uniform. This approach is particularly apt for our laboratory's operations, as we process thousands of sample batches every year, thereby aligning perfectly with the R-QC applicability criteria.

In terms of internal quality control, CV serves as an indicator of a laboratory's testing precision and is an essential component of inter-laboratory quality comparisons [30,31]. Our results indicate that the mean values for the F-QC groups (2.53 ± 0.36, 4.02 ± 0.55, 3.98 ± 0.60) surpassed those for the R-QC groups (2.42 ± 0.25, 3.44 ± 0.46, 3.79 ± 0.55) in all examined parameters (HBsAg, HIV, and TP). Furthermore, the CV percentages for R-QC groups (10.0 %, 13.0 %, 14.0 %) were consistently lower than those for F-QC groups (14.0 %, 14.0 %, 15.0 %). Alarm rates for R-QC groups (4.0 %, 2.0 %) were significantly reduced as compared to F-QC groups (4.7 %, 4.0 %) for HBsAg and HIV items respectively, and the out of control rates of R-QC groups (1.0 %, 0.3 %, 0.0 %) were half or less than those for F-QC groups (2.0 %, 1.0 %, 1.0 %) across all parameters. These findings support the assertion that the R-QC methodology devised in this study not only improves stability but also diminishes the chances of triggering alarms and out of controls. The discrepancy in alarm rates witnessed in the TP group, potentially due to variations in reagent batches, warrants further scrutiny in future studies.

The R-QC method designed in this study is currently applied exclusively within the STAR-VENAS sampling system. Considering the diversity of blood centers and the differences in sampling systems in our country, our next objective is to promote this quality control method to all national blood centers and adapt it to different sampling systems to achieve random sampling of quality control products. In addition, the current random quality control method is only applicable to the four-in-one quality control products containing HBV, HCV, HIV, and TP. In the future, we plan to develop sampling methodologies for multiple combinations of quality control products, enabling randomized sampling across various groups of quality control products.

CRediT authorship contribution statement

Wenping Han: Project administration, Formal analysis. **Jingjing Bao:** Investigation. **Polu Hu:** Software. **Yang Liu:** Validation.

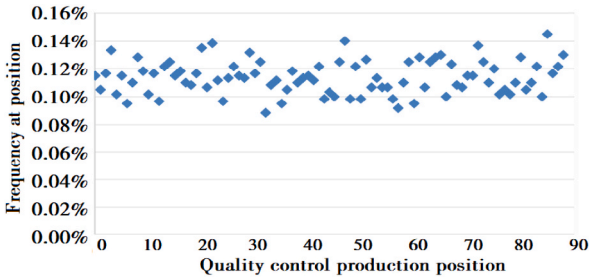

Fig. 5. Frequency of quality control product at position after simulate.

Table 1
Comparison between F-QC and R-QC in items.

Group	n	($\bar{x} \pm s$)	CV (%)	A (%)	L (%)	*F* (a)	*P* (a)
HBsAg (F-QC)	300	2.53 ± 0.36	14.0 %	14 (4.7 %)	6 (2.0 %)	21.7	0.00
HBsAg (R-QC)	300	2.42 ± 0.25	10.0 %	12 (4.0 %)	3 (1.0 %)		
HIV (F-QC)	300	4.02 ± 0.55	14.0 %	12 (4.0 %)	3 (1.0 %)	196.5	0.00
HIV (R-QC)	300	3.44 ± 0.46	13.0 %	6 (2.0 %)	1 (0.3 %)		
TP (F-QC)	300	3.98 ± 0.60	15.0 %	10 (3.3 %)	3 (1.0 %)	16.7	0.00
TP (R-QC)	300	3.79 ± 0.55	14.0 %	11 (3.7 %)	0 (0.0 %)		

Note: n: number of sample batch; CV: coefficient of variation; A: alarm rate; L: out of control rate; (a): Analysis for mean ± standard deviation (x ± s) in items.

Rongrong Pang: Software, Resources. **Rui-ping Dong:** Supervision. **Libo Zhang:** Methodology. **Chengping Ma:** Writing – review & editing, Writing – original draft.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Nanjing Red Cross Blood Center (Approval Letter No.2022-016-03), written consents to participate was acquired from all the patients.

Data availability statement

Data associated with our study had not been deposited in publicly available repositories. Data will be made available on request.

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Declaration of competing interest

The authors declare no competing interests.

Acknowledgement

Not applicable.

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