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RESEARCH ARTICLE

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A method for measuring the experimental resolution of laboratory assays (clinical biochemical, blood count, immunological, and qPCR) to evaluate analytical performance

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Abstract

Background: The measurement method for experimental resolution and related data to evaluate analytical performance is poorly explored in clinical research. We established a method to measure the experimental resolution of clinical tests, including biochemical tests, automatic hematology analyzer methods, immunoassays, chemical experiments, and qPCR, to evaluate their analytical performance.

Methods: Serially diluted samples in equal proportions were measured, and correlation analysis was performed between the relative concentration and the measured value. Results were accepted for $p \leq 0.01$ of the correlation coefficient. The minimum concentration gradient (eg, 10%) was defined as the experimental resolution. For this method, the smaller the value, the higher the experimental resolution and the better the analytical performance.

Results: The experimental resolution of the most common biochemical indices reached 10%, with some even reaching 1%. The results of most counting experiments showed experimental resolution up to 10%, whereas the experimental resolution of the classical chemical assays reached 1%. Unexpectedly, the experimental resolution of more sensitive assays, such as immunoassays was only 25% when using the manual method and 10% for qPCR.

Conclusion: This study established a method for measuring the experimental resolution of laboratory assays and provides a new index for evaluating the reliability of methods in clinical laboratories.

KEYWORDS
analytical performance, biochemical test, experimental resolution, immunoassay, qPCR

1 | INTRODUCTION

Laboratory medicine as a medical discipline plays an indispensable role in predicting disease susceptibility, establishing effective preventive measures, enabling early-stage diagnoses, predicting and monitoring disease, and improving patient-centered care for better prognosis.[1-3] Evaluation of clinical experimental performance is an important prerequisite for ensuring the quality of measurements.[4] The "All Common Checklist" of the College of American Pathologists Accreditation Program stipulates that "for quantitative tests, the

laboratory must verify or establish the method performance specifications that are applicable and clinically relevant".[5] At present, the general performance evaluation indexes include the limit of detection (LoD), accuracy, precision, and linear evaluation.[6,7] However, there is no relevant index that reflects the minimum measurement difference within a certain concentration range. In many cases, the LoD is used to reflect the sensitivity of a detection system. The LoD refers to the smallest concentration that can be reliably measured by an analytical procedure, which can distinguish 0 from the minimum detection concentration but cannot specify the minimum detectable measurement within a certain concentration range.[8,9] Reflecting this experimental minimum within the concentration range is an important performance evaluation index, which is related to, yet different from the LoD. However, at present, there is no relevant evaluation index. Therefore, we introduced the concept of "experimental resolution" to address this issue, with the aim of improving the experimental performance evaluation. The experimental resolution is the minimum change that can be detected by an instrument, which should be the basis of the LoD.[10] Thus, the experimental resolution and LoD are related but separate parameters. As the future of medicine is based on effective patient-centered practice, it is therefore important to select test items with appropriate experimental resolution according to the clinical needs.[11]

Clinical experiments can be divided into quantitative, semi-quantitative, and qualitative assays.[4] The higher the experimental resolution, the better the quantitative effect. Experimental resolution is the key index for evaluating test performance, but no research has been done on a measurement method for experimental resolution or its related data to evaluate test performance.

To address these issues, this study adopted a method involving an equal-proportion dilution series of samples and used the improved linear measurement method to measure the experimental resolution of commonly used assays, including clinical biochemical, automatic hematology analyzer, chemical, immunological, and qPCR assays. By analyzing the test results, we found that the experimental resolution of the clinical biochemical experiments and the automatic hematology analyzer experiments were generally higher than 10% but remained lower than traditional chemical experiments (for which the experimental resolution could reach 1%). Surprisingly, the experimental resolution of the immunoassay and real-time fluorescence quantitative assay, which are generally considered to be more sensitive methods, was lower than 1%.[12-14] By analyzing Pearson's correlation of the correlation coefficients of the results of biochemical samples with different concentration gradients, the results of samples with different dilution ratios could not be predicted, and the experimental resolution should therefore be based on actual measurements rather than relying on a single dilution series. Thus, we propose that the experimental resolution is an important index for the evaluation of experimental performance.

2 | MATERIALS AND METHODS

2.1 | Preparation of equal-proportion dilution samples

For the preparation of samples with a 50% concentration gradient of equal-proportion dilutions, 200 μl normal saline was placed in each Eppendorf (EP) tube, and 200 μl serum was added to the first tube. After thorough mixing, 200 μl diluted sample was taken from the first tube and added to the second tube, which was thoroughly mixed. This was followed by two similar dilutions. Serially diluted samples with relative concentrations of 1000% (undiluted serum), 500%, 250%, 125%, and 62.5% were obtained.

For the preparation of samples with a 25% concentration gradient of equal-proportion dilutions, 200 μl normal saline was placed in each EP tube, and 600 μl serum was added to the first tube. After thorough mixing, 600 μl diluted sample was taken from the first tube and added to the second tube, which was thoroughly mixed. This was followed by two similar dilutions. A series of diluted samples with relative concentrations of 1000% (undiluted serum), 750%, 563%, 422%, and 316% were obtained.

For the preparation of samples with a 10% concentration gradient of equal-proportion dilutions, 160, 80, 40, and 20 μl normal saline were placed in each of the four EP tubes, respectively, and 1440 μl serum was added to the first tube. After thorough mixing, 720 μl diluted sample was taken from the first tube and added to the second tube, which was thoroughly mixed. Then, 360 μl diluted sample was taken from the second tube and added to the third tube, which was thoroughly mixed. Finally, 180 μl diluted sample was taken from the third tube and added to the fourth tube and thoroughly mixed to obtain a series of diluted samples with relative concentrations of 1000% (undiluted serum), 900%, 810%, 729%, and 656%.

For the preparation of samples with a 1% concentration gradient of equal-proportion dilutions, 0.4, 0.2, 0.1, and 0.05 ml normal saline were placed in each of four beakers, respectively, and 39.6 ml serum was accurately measured with an acid burette into the first beaker. After fully mixing, 19.8 ml diluted sample was accurately measured from the first beaker into the second beaker and fully mixed. Then, 9.9 ml diluted sample was taken from the second beaker into the third beaker and thoroughly mixed. Finally, 4.95 ml diluted sample was taken from the third beaker into the fourth beaker and thoroughly mixed to obtain a series of diluted samples with relative concentrations of 1,000% (undiluted serum), 990%, 980%, 970%, and 961%, as shown in Figure 1.

After each serum sample was diluted, albumin (ALB) was measured. If the ALB test results of the diluted samples showed good linearity ($p \leq 0.01$) with the relative concentration, this the dilution was considered accurate, and the sample could be used for subsequent analysis.

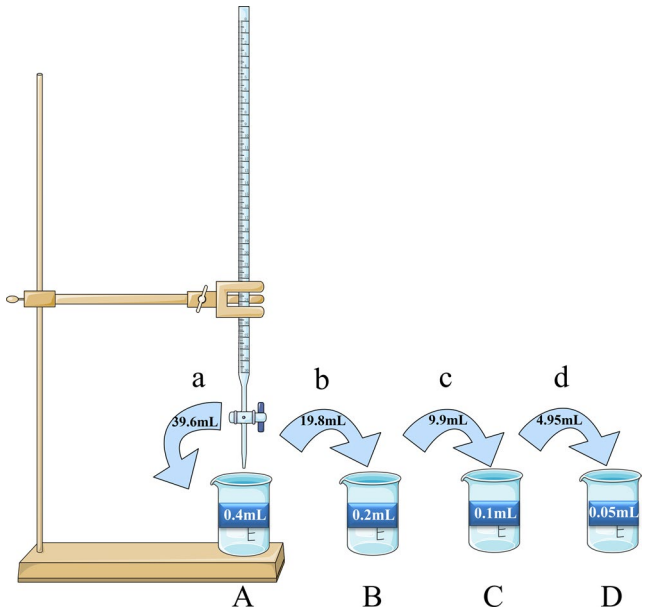

FIGURE 1 Preparation of samples with a 1% concentration gradient of equal-proportion dilutions. To prepare samples, add 0.4, 0.2, 0.1, and 0.05 ml normal saline to four beakers A, B, C, and D, respectively. a. Precisely measure 39.6 ml serum with an acid burette into beaker A, and fully mix. b. Precisely measure 19.8 ml diluted serum from beaker A into beaker B, and thoroughly mix. c. Take 9.9 ml diluted serum from beaker B into beaker C, and mix it thoroughly. d. Take 4.95 ml diluted serum from beaker C into beaker D, and mix it thoroughly

2.2 | Determination of the experimental resolution of biochemical tests, automatic hematology analyzer methods, immunoassays, chemical experiments, and qPCR

2.2.1 | Biochemical tests

In total, 15 items, including glutamic-oxalacetic transaminase (AST), glutamic-alanine transaminase (ALT), total bilirubin (TBil), direct bilirubin (DBil), total protein (TP), albumin (ALB), creatinine (CREA), uric acid (UA), urea (UR), total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), lactate dehydrogenase (LDH), and glucose (Glu) in the diluted serum samples, were detected using a Mindray BS-800 M automatic biochemical analyzer (Shenzhen Mindray Biomedical Electronics Co., Ltd).

2.2.2 | Automatic hematology analyzer method

The Mindray BC-6000 automatic hematology analyzer (Shenzhen Mindray Biomedical Electronics Co., Ltd.) was used to detect nine items, including white blood cells (WBC), neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (EOS), basophils (BAS), red blood cells (RBC), hemoglobin (Hb), and platelets (PLT), in the diluted blood samples.

2.2.3 | Enzyme-linked immunosorbent assay

Serially diluted positive serum samples of anti-HBV surface antigen (anti-HBs) were detected using an anti-HBs commercial ELISA kit (Shenyang Huimin Biological Technology Co., Ltd) according to the manufacturer's instructions.

2.2.4 | Colloidal gold method

The HCG test card was used to detect the HCG-positive series of diluted urine samples, and the reaction results were photographed and processed. Image J v1.8.0 was used to process the photographs to obtain the gray scale of the C area of the quality control line and the T area of the test line. The gray scale ratio (T/C) between the test line and quality control line was calculated using the following formula:

$$T/C = \frac{\text{the gray scale of the C area}}{\text{the gray scale of the T area}} \quad (1)$$

2.2.5 | Chemiluminescence immunoassay

The concentration of carcinoembryonic antigen in the diluted samples was determined using a Mindray i2000 chemiluminescence analyzer (Shenzhen Mindray Biomedical Electronics Co., Ltd.).

2.2.6 | Gas chromatography experiments

A series of diluted toluene and benzene samples were detected by GC-7860 gas chromatography (Shanghai Appropriate Electronic Technology Co., Ltd). The reaction conditions were as follows. The hydrogen pressure in the gas chromatography column was maintained at 0.1 MPa. The chromatographic experimental conditions included a chromatographic column temperature of 80°C, a gasification chamber temperature of 150°C, and a detector temperature of 200°C. Diluted samples of different concentrations were detected, and their complete chromatograms and retention times were recorded. The toluene and benzene contents were calculated as follows:

$$A_i\,\% = \frac{A_i f_{mi}}{\sum A_i f_{mi}} \times 100\,\%$$

where A_i = the peak area of component i, f_{mi} = the relative correction factor of component i, and the relative correction factors of benzene and toluene were 0.89 and 0.94, respectively.

2.2.7 | Flame atomic absorption spectrophotometry

The absorbance values of the copper and strontium diluted samples were determined using an SP-3900AA flame atomic absorption spectrometer (Shanghai Spectrum Instruments Co., Ltd).

2.2.8 | qPCR

TransStart Top Green qPCR Supermix (TransGen Biotech Co., Ltd) and diluted DNA samples were used to construct a qPCR system for the amplification of genes (ie, the 18S ribosomal RNA gene), and the relative DNA concentration N was calculated as follows:

$$N = 2^{(Ct_0 - Ct)} \quad (3)$$

where Ct_0 is the *Ct* value of the undiluted sample and *Ct* is the *Ct* value of the treated sample.

2.3 | Establishment of an experimental resolution method

The linear evaluation method was used to evaluate the experimental resolution. The specific method has been previously reported in the literature,[15] with some modifications:

1. The equal-proportion concentration gradient dilution method was adopted instead of the equal-spacing concentration gradient dilution method for the linear evaluation of the diluted samples.
2. In the original method, the same sample was measured at least twice in parallel. For the purposes of this study, to control the detection range, the same sample was designed to be tested only once.
3. According to the definition of experimental resolution, linear regression was used to analyze the experimental results, with the relative concentration used as the independent variable and the actual test value as the dependent variable for linear fitting. When determining the boundary value, the *p*-value was reduced from 0.05 to 0.01.

Therefore, the modified experimental resolution determination method was as follows: the correlation analysis was conducted between the actual measured values obtained from each experiment and the relative concentration. It was stipulated that the fitting result was valid for $p \leq 0.01$ and invalid for $p > 0.01$. If the fitting result between the detection results and the relative concentration was still valid for the detection of the 50% concentration gradient dilution series samples, then the experimental resolution was 50%—indicating a qualitative experiment; if the fitting result between the detection results and the relative concentration was still valid for the measurement of the 25% concentration gradient dilution series samples, then the experimental resolution was 25%—indicating a semi-quantitative experiment; and if the fitting result between the detection results and the relative concentration was still valid for the measurement of the 10% concentration gradient dilution series samples, then the experimental resolution was 10%—indicating a quantitative experiment. Using urea as an example, the *p*-value of the correlation analysis results between the detection results of the samples with an equal dilution of 50%, 25%, and 10% concentration gradients and the relative concentration <0.01, and the *p*-value of the correlation analysis results between the detection results of samples with equal dilution of a 1% concentration gradient and the relative concentration >0.01, as shown in Figures 2 and 3. The fitting results between the results of the samples diluted in equal proportions up to the 10% concentration gradient and the relative concentration were effective. Therefore, the experimental resolution of urea detection was 10%, which was a quantitative experiment.

3 | RESULTS

The correlation between the detection results of common clinical biochemical indicators of sera with concentration gradients of 25%, 10%, and 1% with the relative concentrations is shown in Table 1. The results showed a significant $p \leq 0.01$ for the correlation test results between the detection results of all items (ALT, AST, TB, DB, TP, ALB, CREA, UA, UREA, TC, TG, HDL, LDL, LDH, Glu) in the samples with a concentration gradient dilution of 25% and the relative concentrations. Except for direct bilirubin ($p = 0.013$), which was greater than the threshold value of 0.01, the correlation test results were significant between all results for the 10% concentration gradient dilution samples, and the relative concentrations ($p \leq 0.01$). For the 1% concentration gradient dilution, only TP, ALB, and UA were significant ($p \leq 0.01$ for all).

The detection results for the experimental resolution of the automatic hematology analyzer are presented in Table S1. The experimental resolutions of BAS and MON could only reach 50% and 25%, respectively. Anticoagulant blood was tested with a 10% concentration gradient dilution, and the test results showed that the *p*-values of WBC, NEU, LYM, EOS, RBC, Hb, and PLT were all within the effective range. In the 1% concentration gradient dilution samples, only the test results for RBC were significant ($p < 0.01$). To judge whether the dilution of a 1% concentration gradient of the anticoagulant was accurate, we used a biochemical method for verification. The results showed that the correlation analysis between the ALB test results, and the relative concentration were significant ($p < 0.01$), which confirmed it as a qualified sample for dilution.

The experimental resolution of the manual immunoassay could only reach 25%, not 10%, while the experimental resolution of the automated immunoassay (chemiluminescence immunoassay) could reach 1%, as shown in Table 2. The experimental resolution detection results of gas chromatography and flame atomic absorption spectrophotometry are shown in Table S2. The results showed that the experimental resolution of both methods reached 1%. The detection results showed that the experimental resolution of qPCR only reached 10%, which was not consistent with the commonly believed high sensitivity, as shown in Table 2.

Pearson's correlation was calculated for the correlation coefficients of the biochemical results of samples with different concentration gradient dilutions. No correlation was detected between the

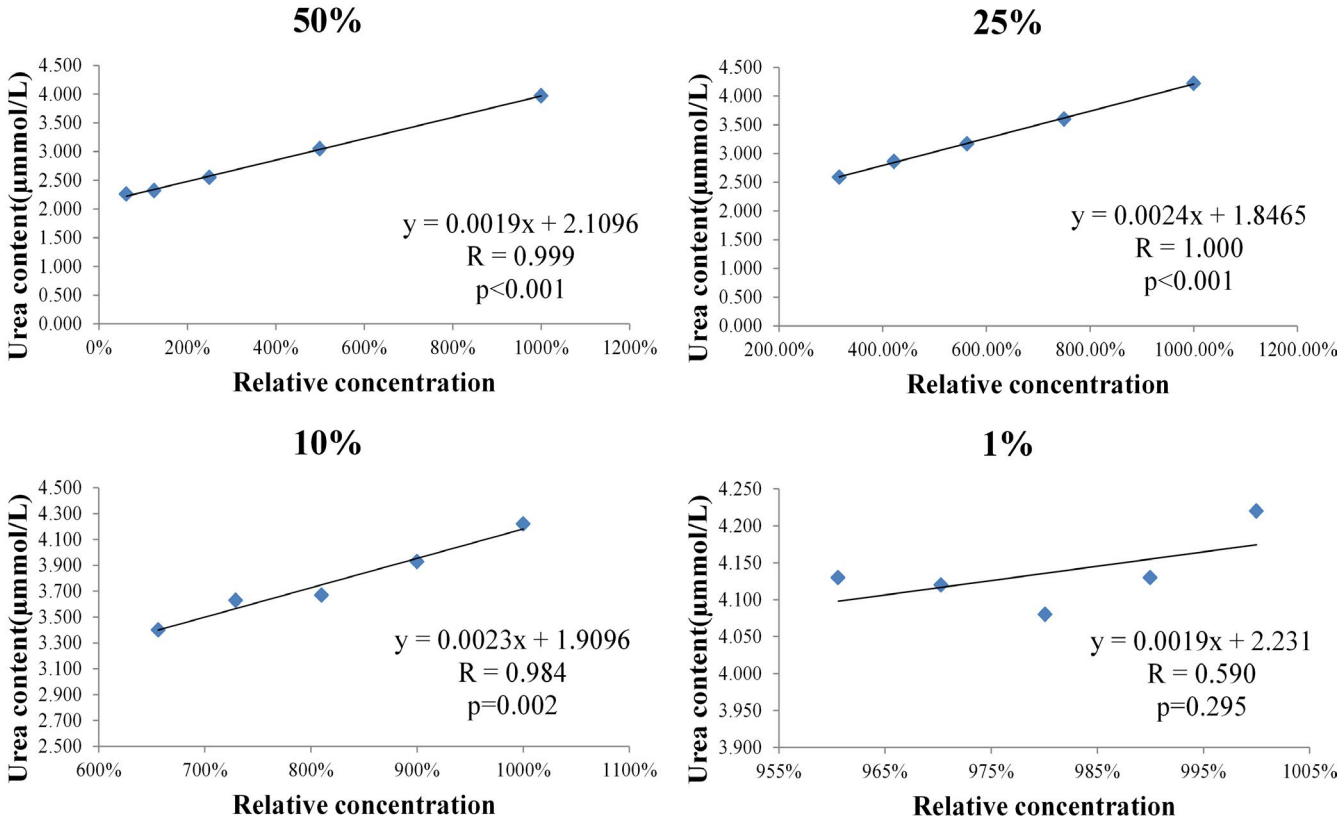

FIGURE 2 Correlation analysis between the test results for serially diluted urea and the relative concentrations

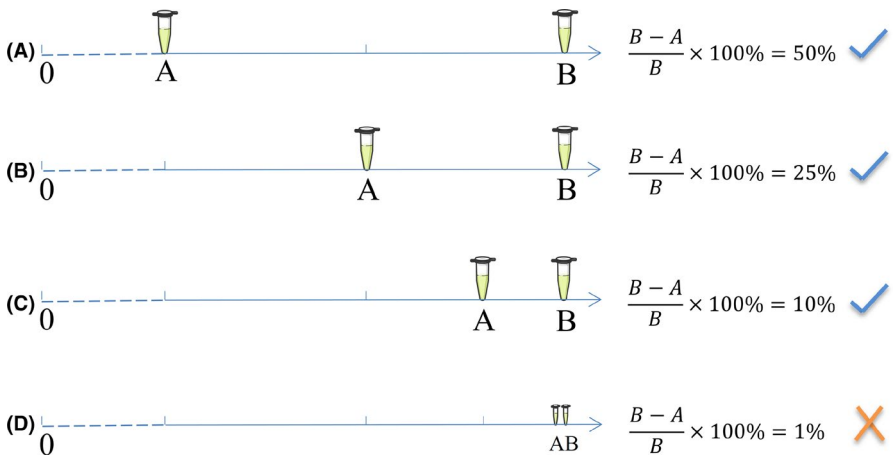

FIGURE 3 Explanation of experimental resolution. Samples with a 1% concentration gradient equal-proportion dilution could not be distinguished, so the experimental resolution of this experiment was 10%. A, The concentration of the lower concentration sample; B, the concentration of the higher concentration sample

results of the samples with 25%, 10%, and 1% concentration gradient dilutions, as shown in Table 3.

4 | DISCUSSION

The experimental resolution refers to the minimum variation that can be detected by an instrument. We believe that the magnitude of the minimum variation can be explained by the merits and demerits of linear fitting. Although imprecision errors or matrix effects can reflect the precision and accuracy of assays, the poor performance of any of the above indicators will affect the experimental resolution. Therefore, the experimental resolution can comprehensively reflect imprecision errors, matrix effects, linearity errors, and other indicators. The experimental resolution is a more intuitive and direct indicator of the advantages and disadvantages of assays compared to the above-mentioned indicators. In clinical practice, specimens are usually tested only once; therefore, experimental resolution advocates that a concentration should also be tested only once, which can be a more rigorous assessment of the experiment, and more in line with clinical practice.

The evaluation of linearity usually adopts the equal-spacing dilution method to dilute samples, such as a group of equal-spacing dilution samples with concentrations of 100, 80, 60, 40, and

TABLE 1 Correlation analysis of the biochemical experiment results and the relative concentrations

	25%						10%	
Analytes	**1000%**	**750%**	**563%**	**422%**	**316%**	**R (P)**	**1000%**	**900%**
ALT, U/L	11.900	9.000	5.600	5.200	4.100	0.984 (0.002)	**28.800**	**26.400**
AST, U/L	16.400	12.600	10.400	7.000	4.800	0.992 (0.001)	**18.600**	**16.800**
TBil, μmol/L	8.490	6.240	4.430	3.180	2.110	1.000 (<0.001)	**11.990**	**10.770**
DBil, μmol/L	–	–	–	–	–	–	**3.210**	**3.150**
TP, g/L	74.200	54.800	41.000	29.400	21.600	1.000 (<0.001)	74.200	66.800
ALB, g/L	48.900	37.200	28.200	20.800	15.400	0.999 (<0.001)	49.200	44.300
CREA, μmol/L	74.900	60.800	48.100	41.900	36.500	0.998 (<0.001)	**59.400**	**55.700**
UA, μmol/L	345.100	256.200	187.400	136.500	102.600	1.000 (<0.001)	345.100	307.200
UREA, mmol/L	4.220	3.600	3.170	2.860	2.590	1.000 (<0.001)	**4.220**	**3.930**
TC, mmol/L	4.820	3.950	2.720	2.050	1.530	0.994 (<0.001)	**5.150**	**4.560**
TG, mmol/L	0.610	0.490	0.370	0.290	0.220	0.998 (<0.001)	**1.310**	**1.180**
HDL, mmol/L	1.890	1.430	1.050	0.780	0.590	1.000 (<0.001)	**2.080**	**1.900**
LDL, mmol/L	1.990	1.490	1.110	0.830	0.610	1.000 (<0.001)	**2.680**	**2.410**
LDH, U/L	138.100	105.400	78.700	58.300	44.200	1.000 (<0.001)	**138.100**	**126.700**
Glu, mmol/L	4.680	3.510	2.590	1.910	1.430	1.000 (<0.001)	**4.660**	**4.160**

TABLE 2 Correlation analysis of the ELISA, colloidal gold assay, chemiluminescence immunoassay, and qPCR results with the relative concentrations

	50%			25%		
Assays	**Relative concentration**	**Measured value**	**R (P)**	**Relative concentration**	**Measured value**	**R (P)**
ELISA	1000%	1.204	0.987 (0.002)	1000%	**0.989**	**0.978 (0.004)**
	500%	0.810		750%	**0.631**	
	250%	0.490		563%	**0.192**	
	125%	0.302		422%	**0.092**	
	63%	0.184		316%	**0.056**	
The colloidal gold assay	1000%	1.513	0.996 (<0.001)	1000%	**1.513**	**0.975 (0.005)**
	500%	1.146		750%	**1.249**	
	250%	0.860		563%	**0.906**	
	125%	0.751		422%	**0.878**	
	63%	0.706		316%	**0.812**	
The chemiluminescence immunoassay, ng/ml	-	-	-	1000%	48.460	0.988 (0.002)
	-	-		750%	41.660	
	-	-		563%	33.090	
	-	-		422%	25.490	
	-	-		316%	18.960	
qPCR	1000%	1.000	0.994 (0.001)	1000%	1.000	0.988 (0.002)
	500%	0.420		750%	0.547	
	250%	0.107		563%	0.382	
	125%	0.056		422%	0.222	
	63%	0.025		316%	0.121	

20 mmol/L.[16,17] However, the problem with this method is the degree of change between the sample concentrations before and after dilution. For example, when considering a difference of 25 mmol/L between samples, the concentration changed by 30% when a 75 mmol/L sample was diluted to 50 mmol/L, whereas the concentration changed by 50% when a 50 mmol/L sample was diluted to

				1%					
810%	**729%**	**656%**	**R(P)**	**1000%**	**990%**	**980%**	**970%**	**961%**	**R (P)**
25.600	**22.900**	**22.500**	**0.982 (0.003)**	11.900	11.100	11.500	10.700	11.300	0.569 (0.317)
15.600	**14.100**	**12.100**	**0.993 (0.001)**	16.400	15.200	16.100	15.600	15.700	0.343 (0.572)
9.650	**8.690**	**7.700**	**1.000 (<0.001)**	8.550	8.270	8.540	8.520	8.780	0.618 (0.267)
2.880	**2.910**	**2.730**	**0.952 (0.013)**	3.460	3.300	3.480	3.480	3.460	0.370 (0.540)
59.800	53.400	47.900	1.000 (<0.001)	**74.200**	**72.400**	**71.800**	**71.300**	**70.300**	**0.971 (0.006)**
40.600	36.500	33.300	1.000 (<0.001)	**46.600**	**46.100**	**45.800**	**45.100**	**44.400**	**0.988 (0.001)**
50.500	**45.500**	**42.200**	**0.996 (<0.001)**	74.900	74.200	73.600	72.400	72.800	0.935 (0.020)
276.200	248.000	221.400	1.000 (<0.001)	**345.100**	**337.800**	**334.900**	**330.100**	**329.600**	**0.964 (0.008)**
3.670	**3.630**	**3.400**	**0.984 (0.002)**	4.220	4.130	4.080	4.120	4.130	0.590 (0.295)
4.150	**3.720**	**3.390**	**0.999 (<0.001)**	5.000	5.000	5.030	4.920	4.850	0.811 (0.096)
1.070	**0.960**	**0.860**	**1.000 (<0.001)**	1.320	1.330	1.330	1.300	1.280	0.799 (0.105)
1.730	**1.560**	**1.390**	**0.998 (<0.001)**	1.170	1.200	1.190	1.170	1.150	0.563 (0.323)
2.180	**1.960**	**1.740**	**0.999 (<0.001)**	3.350	3.370	3.410	3.420	3.280	0.249 (0.686)
114.900	**103.300**	**88.900**	**0.994 (<0.001)**	138.100	136.200	136.800	132.800	132.800	0.915 (0.029)
3.760	**3.380**	**3.040**	**1.000 (<0.001)**	4.680	4.600	4.580	4.570	4.500	0.954 (0.012)

10%			**1%**		
Relative concentration	**Measured value**	**R (P)**	**Relative concentration**	**Measured value**	**R (P)**
1000%	0.989	0.854 (0.065)	–	–	-
900%	0.284		–	–	
810%	0.091		–	–	
729%	0.069		–	–	
656%	0.067		–	–	
1000%	1.513	0.948 (0.014)	–	–	-
900%	1.201		–	–	
810%	1.173		–	–	
729%	1.040		–	–	
656%	0.994		–	–	
1000%	48.460	0.958 (0.010)	1000%	**32.870**	**0.959 (0.010)**
900%	48.680		990%	**31.110**	
810%	44.650		980%	**31.150**	
729%	42.000		970%	**29.620**	
656%	39.100		961%	**29.250**	
1000%	**1.000**	**0.996 (<0.001)**	1000%	1.000	0.224 (0.717)
900%	**0.774**		990%	0.774	
810%	**0.607**		980%	0.633	
729%	**0.493**		970%	0.763	
656%	**0.361**		961%	0.908	

25 mmol/L; therefore, the equal-proportion dilution method must be used. Unlike testing the linearity of an experiment using a single concentration gradient dilution sample, we designed an experiment using the equal-proportion dilution method, which ensured that the concentration of each point in the dilution process changed to the same degree, so that the experimental resolution could be

TABLE 3 Pearson's correlation between the correlation coefficients of the biochemical results of samples and the different concentration gradient dilutions

Analytes	25%	10%	1%
ALT	0.984	0.982	0.568
AST	0.992	0.992	0.344
TBil	1.000	1.000	0.617
DBil	–	0.952	0.370
TP	1.000	1.000	0.971
ALB	0.999	0.999	0.988
CREA	0.998	0.996	0.935
UA	1.000	1.000	0.964
UREA	1.000	0.984	0.590
TC	0.994	0.999	0.811
TG	0.998	1.000	0.799
HDL	1.000	0.998	0.563
LDL	1.000	0.999	0.249
LDH	1.000	0.994	0.915
Glu	1.000	1.000	0.954
Pearson's correlation coefficient (P)	−0.890 (<0.001)	0.482 (0.069)	

accurately determined. A qualitative experiment divided an item into two categories (positive or negative).[18,19] Therefore, we believe that an experiment can only be used for qualitative analysis when the experimental resolution reaches 50%. In contrast, a quantitative experiment divides an item into at least 10 parts; thus, we believe that an experiment can only be used for quantitative analysis when the experimental resolution reaches 10%. If the experimental resolution reaches 25%, we believe that the experiment can be used for semi-quantitative analysis.[20] Based on the above principles, we designed a method to measure experimental resolution and applied our method to analyze various experimental procedures, including an immunoassay, chemical assay, automatic hematology analyzer assay, and real-time fluorescence quantitative PCR. Our experiments revealed that $p \leq 0.05$ was not an ideal test threshold; hence, according to the EP6-A Guidelines of the National Committee for Standardization of Clinical Laboratories of the United States, we propose that $p \leq 0.01$ is a more appropriate test threshold.[15]

The results showed that the experimental resolution of assaying direct bilirubin only reached 25%, indicating that this assay could only be used for semi-quantitative analysis, whereas the experimental resolution of the other biochemical experiments, including ALT, AST, TB, TP, ALB, CREA, UA, UREA, TC, TG, HDL, LDL, LDH, and Glu, all reached 10%, indicating that these assays could be used for quantitative analysis. Among them, the experimental resolution of TP, ALB, and UA reached 1%, indicating that they could be used for more accurate measurements. As for blood cell counts, the experimental resolution of macrophage detection was only 25%, and that of basophil detection only 50%, due to the small number of macrophages and basophils. The experimental resolution of other assays, such as WBC, NEU, LYM, EOS, HB, and PLT, reached 10%, indicating that they could be quantitatively detected, while the experimental resolution of RBC detection reached 1% and is therefore a more accurate assay. It is generally believed that the sensitivity of immune experiments is high, but we found that the experimental resolution of manual immune experiments only reached 25%—not 10%.[21] Therefore, manual immune experiments only achieved semi-quantitative analysis, while automated immune experiments reached 1%, allowing for quantitative analysis. The biochemical experiment was based on a chemical assay, but it achieved a better experimental effect.[22] Therefore, we further explored the classical chemical experiments. The results showed that the experimental resolution of both gas chromatography and flame atomic absorption spectrophotometry reached 1%, indicating that classical chemical methods remain effective methods of measurement. The results showed that the experimental resolution of qPCR was 10%—not 1%, which was contrary to the high sensitivity usually associated with this method. By calculating Pearson's correlation of the correlation coefficients for the biochemical results of samples with different concentration gradients, we found no mutual prediction effect between the results of different dilution series, and the experimental resolution should therefore be based on the actual measurement. Furthermore, it was not possible to use only one dilution series to predict higher experimental resolution results (see Table 3). In addition, for the fitting curve, we only considered whether the fitting result met the requirement of $p \leq 0.01$ and did not consider the slope or intercept of the fitting curve. If the fitting effect is good, the accuracy can be further improved by regression.

In conclusion, the established determination method for experimental resolution effectively detected the experimental resolution of various clinical experiments and can be used to evaluate whether biochemical methods, automatic hematology analyzer methods, immunoassay methods, chemical methods, and qPCR are qualitative, semi-quantitative, or quantitative experiments, which have significant implications for the evaluation of clinical trial performance. Therefore, the experimental resolution may be considered as a new index for the performance evaluation of clinical trials, which will influence new discoveries resulting from biochemical tests, complete blood count tests, chemical experiments, immunoassays, qPCR, and other medical tests.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

AUTHOR CONTRIBUTIONS

Hui Liu and Chenxi Sun designed the experiments. Chenxi Sun and Dongxia Wang performed the experiments. Chenxi Sun analyzed the data and wrote the study. Hui Liu reviewed and edited the study. Henggui Xu, Guang Yang, and Xiaomei Yan provided study materials. All authors have read and approved the final study and take responsibility for its integrity.

DATA AVAILABILITY STATEMENT

All relevant data are within the study, and no additional data are available.

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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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