RESEARCH ARTICLE

WILEY

Investigation of the quantitative detection of serum Helicobacter pylori antibody in clinical laboratories in China

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Abstract

Background: This study aimed to investigate the implementation and quality control of the quantitative detection of serum Helicobacter pylori (H. pylori) antibody in clinical laboratories in China.

Methods: Online external quality assessment (EQA) questionnaires were distributed to the clinical laboratories by National Center for Clinical Laboratories (NCCL) of China. We collected information on the quantitative detection procedures of serum H. pylori antibody in clinical laboratories, including detection reagents, methods, instruments, calibrators, and internal quality control (IQC). We distributed quality control products to some select laboratories that conducted quantitative detection and analyzed the obtained test data. We evaluated the quantitative detection procedure based on the standard evaluation criteria set at a target value of ±30%.

Results: 70.9% (146/206) of the laboratories conducted quantitative detection of H. pylori antibody; 29.1% (60/206) of the laboratories performed qualitative detection. Domestic reagents and matching calibrators accounted for more than 97.1% (200/206) of all reagents. Latex-enhanced immunoturbidimetry was used in 89.7% (131/146) of the laboratories for quantitative determination, while the colloidal gold method was used in 66.7% (40/60) of the laboratories for qualitative determination. A total of 130 laboratories participated in the EQA; 123 completed the assessment, and the pass rate was 75.6% (93/123).

Conclusion: Clinical quantitative detection of serum H. pylori antibody is performed at a high rate in China. Thus, further studies on the specificity of commercial detection reagents are needed. EQAs are useful to monitor and improve the detection quality of H. pylori antibodies.

KEYWORDS
external quality assessment, Helicobacter pylori antibody, internal quality control, quantitative detection, serum

1 | INTRODUCTION

Helicobacter pylori (H. pylori) infection is a high-risk factor for gastric cancer. A previous study reports huge regional differences among with more than half of the world's population infected.[1] East Asia, particularly Japan, South Korea and China, and western Europe have higher rates of gastric cancer as compared to other regions.[2-4] In China, the annual prevalence and mortality due to gastric cancer are more than twice the global average.[5-7] In China, annually, there are 679,100 new cases and 498,000 gastric cancer-associated deaths; these account for 23% of all deaths from malignant tumors.[8] It accounts for 50% of deaths associated with tumors of the digestive system. The current proportion of gastric cancer patients under 30 years of age has risen to 3.3% from 1.7% in the 1970s.[7,9,10]

A retrospective, cross-sectional study of H. pylori infection in a community of Hebei province (4,796 subjects) showed the infection prevalence at 52.3%.[3] A study focused on senior citizens (>60 years) in Beijing, China, found that the infection prevalence was 83.4%.[11] In China, a survey of H. pylori infections is conducted in areas with high incidences of gastric cancer. Among 5,417 healthy individuals aged between 0 and 69 years, the prevalence of H. pylori infection was at 63.4%.[12] The incidences of H. pylori infections are closely related to the socio-economic levels, population density, public health conditions and water supply.[2,13,14] Children living in poor socio-economic conditions had a higher risk of H. pylori infection. Although the mode of transmission of the infection remains unknown, interpersonal transmission appears to be the main route.[11,12,15] The H. pylori infection rate in the natural population of China is 40%–90% (average 59%); the lowest is in Guangdong (42%), and the highest is in Xizang (90%) provinces.[16-18]

Because of the pathogenicity and the prevalence of H. pylori infection in the population, improving the detection and diagnosis methods is important. In the last few years, significant advances have been made in both physical (endoscopy) and molecular approaches. In Table 1, we have summarized the advantages and disadvantages of different detection methods for H. pylori infection.

The accuracy of the results of clinical laboratory directly determines the reliability and efficiency of disease diagnosis and treatment, respectively. Therefore, it is very important to ensure the accuracy and the comparability of results between different methods and in different laboratories. External quality assessment (EQA) is a process in which the same specimens are analyzed by multiple laboratories, and the reported results are collected, evaluated, and compared by an external independent agency. The NCCL of China determines the calibration and detection capabilities through inter-laboratory comparisons and monitors the progress of the laboratories. Therefore, EQA (proficiency testing, PT) could guarantee the accuracy and comparability of the results.

2 | MATERIAL AND METHODS

2.1 | Respondents

The respondents included laboratories that volunteered to participate in the EQA survey for quantitative detection of serum H. pylori antibody, 2020.

2.2 | Questionnaire survey

The clinical laboratories responded to the questionnaire on "Investigations on the Quantitative Detection of H. pylori Antibody" through the EQA system of the NCCL of China. In addition to the basic information, the questionnaire contained eight questions, including whether the laboratory carried out quantitative detection of H. pylori antibody, specimen type, detection method, reagent brand, instrument brand, calibration product brand, the concentration unit, and their willingness to participate in the EQA survey (serum matrix) for quantitative detection of H. pylori antibody conducted by the NCCL of China, 2020 (Table S1). The information was collected and the general situation on the quantitative detection of H. pylori antibody in the clinical laboratories was summarized.

2.3 | External quality assessment survey

We issued the EQA investigation notice and application entry through the NCCL of China. We distributed quality control products to the laboratories that had applied. There were three H. pylori antibody samples with different concentrations, numbered 202011, 202012, and 202013. The concentration covers cutoff, medium, and high values and all concentrations were within the linear detection range of each brand of instruments.

2.4 | External quality assessment evaluation criteria

Evaluation criteria were set at a target value of ±30%; the target value represented the statistically robust mean value after grouping. If the deviation of the three specimens was within the range, the detection result was considered as "qualified", else, it was "unqualified."

2.5 | Statistical analysis

According to the feedback and the reported results, the total number and percentages of the responses in the questionnaire were calculated. The quantitative detection results of the H. pylori antibodies were grouped according to reagents and evaluated. Each group was

TABLE 1 The advantages and disadvantages of different detection methods of Helicobacter pylori infection

Method	Advantages	Limitations	Reliability	Cost	Operability	Clinical applicability
Culture	Gold standard Specific 100%	Time-consuming Low sensitivity Complex operation	Reliable	High	Scientific research Less clinical	Drug sensitivity test
Rapid Urease Test, RUT	Fast Specific Sensitive	Time-consuming Invasive Focally distribution	Reliable Qualitative	High	Inconvenient Need fasting Painful	Infection confirmed (Gastroscopy population)
Urea Breath Test, UBT	Simple operation Specific Accuracy	Drug effects Expensive Radioactivity	Relatively reliable Quantifiable.	Relatively High	Easy Not applicable to the population	Infection confirmed (Physical screening population) Bactericidal effect judgment
Stool antigen test, SAT	Accuracy Easy to get	Easy pollution, Manual operation, Poor sensory	Relatively reliable Qualitative	Medium	Manual sampling Sample pretreatment	Infection detection (Children and special population) Antibacterial efficacy judgment
Colloidal gold	Convenient Fast	Low sensitivity Cannot distinguish past and present infection Reagent validation	Qualitative Missed diagnosis	Low	Easy Serum Subjective judgment	Preliminary screening
Western blotting	Specific Strain typing Guiding medication	Unsuitable for screening Cannot distinguish past and present infection	Qualitative Missed diagnosis	Low	Easy Serum Subjective judgment	Guiding medication
Biochemical antibodies	Accuracy Batch detection Automatic	Cannot distinguish past and present infection	Reliable Quantitative Traceability	Low	Easy Serum Automatic judgment	Preliminary screening Epidemiological investigation Screening of inpatients Methodological supplements

named as reagents A, B, and C. When no fewer than two laboratories existed in one group, the mean value, coefficient of variation (CV), and bias between the mean and target values of that reagent group were calculated.

3 | RESULTS

3.1 | Composition ratios of detection methods

Several detection methods are used in clinical laboratories. In the questionnaire, we evaluated the proportion of current quantitative and qualitative detections and the proportion of the various detection methods under the two. The results showed that the quantitative detection of serum H. pylori antibodies accounted for about 71%, while the qualitative detection accounted for about 29% of total detections. Thus, the proportion of quantitative detection is relatively higher; the proportion of immunoturbidity in quantitative detection was at 89.7%. Other detection methods, including chemiluminescence immunoassay (CLIA), quantum dots-based immunofluorescence (QD-IF), immunity transmission turbidity (ITA), and fluorescence immunochromatography assay (FICA), accounted for approximately 10% of the total detection methods. Among the qualitative detection methods, the colloidal gold method was the most commonly used technique, accounting for approximately 66.7%, while other methods including western blotting, colloidal gold immunochromatography, pH indicator, and ELISA, accounted for approximately 33.4% of the total estimate (Table 2).

3.2 | Group statistics based on detection methods

We set three concentrations for controls as follows: the cutoff value, the middle value, and the high value. Since this investigation was mainly focused on quantitative detection, the quality control materials were only issued to the laboratories which performed quantitative detection. Different clinical laboratories used different instruments, methods, and reagents. Table 3 shows the group statistics based on the different detection methods. Laboratories that did not specify their detection methods were grouped as "other group." As shown in Table 3, we were unable to calculate the robust standard deviation (SD), standard uncertainty, and robust CV, all based on ISO13528 standards for only one laboratory. The results showed that there were significantly high differences in the robust means between different detection methods, which indicated the necessity of grouped statistics. For the detection methods, in quantum dots-based immunofluorescence, as the concentration of quality control substance increased, the robust CV also increased; this may be due to the defect in the detection method, and small sample size. In general, the robust CV of other groups was also high which may be attributed to the small sample size, and different detection methods in the other groups.

3.3 | Group statistics based on detection reagents

In addition to detection methods, we also grouped the responses based on detection reagents. Because the same detection method may utilize reagents from different suppliers, grouping by reagents could be more accurate. As shown in Table 4, after grouping by reagents, the robust CV, on the whole, was smaller than that obtained on grouping by detection method. Among these, reagent C accounted for the largest market share, which suggested that reagent C was used in most Chinese clinical laboratories for quantitative detection of H. pylori antibodies. The robust SD of the reagent C group was also relatively lesser, which suggested that increasing the sample size of laboratories involved could result in more accurate statistical interpretations. Although both reagents A and C were used in the latex-enhanced immunoturbidimetry method, the results were quite different. On the one hand, it suggested the necessity for grouping. On the other hand, it also suggested that the commutability of quality control methods may also need improvement.

3.4 | Group statistics based on grouping principle

Based on the above statistical results, it could be concluded that detection reagents and methods are used in a complete set. That is, a certain manufacturer's reagent is generally matched to its unique detection method. The NCCL of China, as an EQA institution of clinical laboratories, has a default grouping principle for evaluating the results. Regardless of the grouping statistics based on instruments, methods, or reagents, we grouped based on the criterion of the number of participating laboratories; greater than or equal to 18 or 12 were in one group under ISO 13528, else they were grouped as "other" group. The results were analyzed based on the robust mean, robust SD, and robust CV calculated for all the participating laboratories according to ISO 13528. We found that, in this survey, due to the limited number of participating laboratories, this was not a suitable criterion for grouping. Therefore, on the premise of grouping according to reagents, we classified the number of participating laboratories as greater than or equal to 5 into separate groups. The results are shown in Table 5. According to the above grouping principle, robust CV values fluctuated less. Since reagent C was used by many participating laboratories in the survey, accounting for approximately 84.5% of the total number of laboratories, the robust mean, robust SD, and standard uncertainty of all laboratories were close to those of the reagent C group. When the EQA of quantitative detection of serum H. pylori antibody is formally conducted, this effect can be minimized by increasing the number of participating laboratories.

3.5 | Pass rates of grouped statistics

All participating laboratories were grouped and analyzed based on the reagents used, and the results were evaluated based on the

TABLE 2 The proportion of various qualitative and quantitative detection methods

	Method	Amount	Proportion
Quantitative detection	Chemiluminescence immunoassay, CLIA	4	2.7%
	Latex-enhanced immunoturbidimetry	131	89.7%
	Quantum dots-based immunofluorescence, QD-IF	5	3.4%
	Immunity transmission turbidity, ITA	4	2.7%
	Fluorescence immunochromatography assay, FICA	2	1.4%
Qualitative detection	ELISA	7	11.7%
	PH indicator	1	1.7%
	Colloidal gold	40	66.7%
	Colloidal gold immunochromatography	2	3.3%
	Western blotting	10	16.7%

TABLE 3 The result of grouped statistics according to detection methods

Batch number	Group	Total number	Robust mean	Robust standard deviation	Standard uncertainty	Robust CV%
202011	All	123	12.85	1.34	0.151	10.46
	Chemiluminescence immunoassay, CLIA	1	39.18	--	--	--
	Latex-enhanced immunoturbidimetry	112	12.71	1.3	0.154	10.26
	Fluorescence immunochromatography assay	1	197.9	--	--	--
	Immunity transmission turbidity, ITA	4	13.64	1.37	0.856	10.05
	Quantum dots-based immunofluorescence	2	18.13	0.55	0.489	3.05
	Others	3	14.11	2.81	2.026	19.9
202012	All	123	23.89	2.4	0.271	10.05
	Chemiluminescence immunoassay, CLIA	1	63.41	--	--	--
	Latex-enhanced immunoturbidimetry	112	23.7	2.21	0.261	9.32
	Fluorescence immunochromatography assay	1	272.7	--	--	--
	Immunity transmission turbidity, ITA	4	24.33	1.85	1.154	7.59
	Quantum dots-based immunofluorescence	2	32.45	3.82	3.374	11.76
	Others	3	23.11	2.81	2.026	12.15
202013	All	123	44.14	6.5	0.733	14.73
	Chemiluminescence immunoassay, CLIA	1	80.46	--	--	--
	Latex-enhanced immunoturbidimetry	112	43.69	6.12	0.723	14.01
	Fluorescence immunochromatography assay	1	335.6	--	--	--
	Immunity transmission turbidity, ITA	4	46.71	7.74	4.838	16.57
	Quantum dots-based immunofluorescence	2	79.84	16.48	14.565	20.64
	Others	3	41.79	7.3	5.268	17.47

target value of ±30%. The target value is a robust average value, and ±30% is the allowed degree of dispersion of the detection results. If the detection value was within this range, the result was considered acceptable; if the detection value was outside this range, the result was considered unacceptable. The results are shown in Table 6. In the low-concentration group, the pass rate for all laboratories using other reagents was 57.1%; the pass rate for the reagent C group was 88.5%, and the pass rate for the reagent A group was 100%. In the medium concentration group, the pass rate for all laboratories using other reagents was 42.9%; for the reagent C group it was 87.5%, and for the reagent A group was 83.3%. In the high concentration group, the pass rate for all laboratories using other reagents was 28.6%;

the pass rate for the reagent C group was 89.4%, and the pass rate for reagent A group was 91.7%. It could be concluded that with the increase in antibody concentration, the pass rate of other reagent groups declined. The pass rate of the reagent C group was basically the same (88.5%, 87.5%, and 89.4%). The pass rate of the reagent A group at low and high concentrations was higher than the reagent C group (100% vs. 88.5%; 91.7% vs. 89.4%), but at medium concentration group, the pass rate of the reagent A was lower the that of the reagent C (83.3% vs. 87.5%). Based on this result, we cannot arbitrarily judge whether reagent A or C is better, perhaps more accurate statistics can be obtained by increasing the number of participating laboratories.

TABLE 4 The result of grouped statistics according to detection reagents

Batch number	Group	Total number	Robust mean	Robust standard deviation	Standard uncertainty	Robust CV
202011	All	123	12.85	1.34	0.151	10.46
	Reagent A	12	133.21	15.01	5.418	11.27
	Reagent B	1	13.67	--	--	--
	Reagent C	104	12.54	1.05	0.128	8.34
	Reagent D	1	39.18	--	--	--
	Reagent E	1	197.9	--	--	--
	Reagent F	1	1.94	--	--	--
	Reagent G	2	18.13	0.55	0.489	3.05
	Reagent H	1	3.5			
202012	All	123	23.89	2.4	0.271	10.05
	Reagent A	12	185.77	7.46	2.693	4.02
	Reagent B	1	25.69	--	--	--
	Reagent C	104	23.31	1.73	0.212	7.41
	Reagent D	1	63.41	--	--	--
	Reagent E	1	272.7	--	--	--
	Reagent F	1	2.64	--	--	--
	Reagent G	2	32.45	3.82	3.374	11.76
	Reagent H	1	5.2	--	--	--
202013	All	123	44.14	6.5	0.733	14.73
	Reagent A	12	241.19	21.69	7.826	8.99
	Reagent B	1	58.41	--	--	--
	Reagent C	104	42.71	5.2	0.638	12.18
	Reagent D	1	80.46	--	--	--
	Reagent E	1	335.6	--	--	--
	Reagent F	1	9.11	--	--	--
	Reagent G	2	79.84	16.48	14.565	20.64
	Reagent H	1	6.5	--	--	--

TABLE 5 The result of grouped statistics according to the grouping principle

Batch number	Group	Total number	Robust mean	Robust standard deviation	Standard uncertainty	Robust CV
202011	All	123	12.85	1.344	0.151	10.46
	Reagent C	104	12.54	1.046	0.128	8.34
	Reagent A	12	133.21	15.014	5.418	11.27
202012	All	123	23.89	2.401	0.271	10.05
	Reagent C	104	23.31	1.728	0.212	7.41
	Reagent A	12	185.77	7.463	2.693	4.02
202013	All	123	44.14	6.503	0.733	14.73
	Reagent C	104	42.7	5.202	0.638	12.18
	Reagent A	12	241.19	21.688	7.826	8.99

4 | DISCUSSION

External quality assessment, also known as "proficiency testing", is used to evaluate the laboratory testing ability. It is an important method to identify the problems in the clinical laboratory and design necessary interventions. EQA is an important external monitoring tool for quality assurance, especially when there is neither a reference method nor a reference material. In recent years, several new

TABLE 6 The passing rate of grouped statistics

Batch number	Group	Total number	Number of passing laboratories	Pass rate%
202011	Reagent others	7	4	57.1%
	Reagent C	104	92	88.5%
	Reagent A	12	12	100%
202012	Reagent others	7	3	42.9%
	Reagent C	104	91	87.5%
	Reagent A	12	10	83.3%
202013	Reagent others	7	2	28.6%
	Reagent C	104	93	89.4%
	Reagent A	12	11	91.7%

protein detection indicators have been utilized for clinical testing. The common characteristic of these kit-based testing is the domination of domestic reagent manufacturers. On the one hand, it contributes to the rapid development of the medical diagnostic industry in China; on the other hand, it also reflects the importance of conducting the corresponding EQA program. Thus, in 2020, The NCCL of China conducted an EQA survey for the quantitative detection of H. pylori antibodies.

The EQA survey in China showed that the number of clinical laboratories for quantitative detection of H. pylori antibodies was twice that for qualitative detection. In the quantitative detection, the proportion of latex-enhanced immunoturbidimetry method was the highest, accounting for approximately 90%, while in the qualitative detection, the colloidal gold method accounted for approximately two-thirds of the total. This indicated that although there are several detection methods, they are relatively concentrated. Upon analyzing the reported data, we found that the results were significantly different depending on the detection methods used, even in different orders of magnitude (Table 3). However, on using the same detection method but different reagents, the detection value can vary by 6- to 10-fold as the concentration changes from high to low (Table 4, Reagents A and C). Even with the same reagent and the same method, the numerical difference between different laboratories varied by more than three times (Reagent C). Reagent C is a commonly used domestic reagent for H. pylori antibody detection in clinical laboratories of China. The calibrators are in combination with foreign-manufactured analyzers. In general, according to the current requirements for medical device registration, the analysis system composed of the reagents and their calibration products produced by the reagent manufacturers and the "applicable instruments" on the kit instructions are a supporting system, which can be termed as the "open" supporting system. Correspondingly there are closed systems, including Roche reagents, calibrators, and Cobas automatic biochemical analyzer analysis system. The reasons for the large differences in results for the "open" matching systems are complex. One possible reason could be the design of different analysis platforms, including the settings for absorbance wavelength, data reading methods, and the built-in calibrations. It is impossible to delineate the single factors from the present EQA data.

We found that a considerable number of laboratories were (or will be) conducting quantitative detection of H. pylori antibodies, and the latex immunoturbidimetric method would be a common method. Although the pass rate of the quantitative detection of H. pylori antibody was relatively high in this survey, it does not imply that the quality of this indicator met the clinical requirements. This is because the evaluation standard of target value set at ±30% is relatively less stringent. There are several limitations for EQA, including the wrong number entry, the wrong sample sequence, and other handling errors, such as IQC. The large IQC and CV also contributes as one of the main factors. The quality control products used for investigation were derived from human serum, which may also contain a variety of other antibody proteins in addition to the H. pylori antibodies. The large differences in the test results between laboratories may be due to the reagents, methods, and properties, including specificity and anti-interference effects.

5 | CONCLUSION

To the best of our knowledge, this is the first report from China where the status of quantitative detection of serum H. pylori antibody was investigated. Although the number of laboratories included in the survey was limited, some problems in the quantitative detection of H. pylori antibodies have been reflected in our results. Commercially available quantitative detection kits need methodological research, and the quality control measures need to be improved. This study laid a foundation for the development of a formal EQA for the detection of serum H. pylori antibodies for future research.

CONFLICT OF INTEREST
The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

Chao Zhang was involved in acquisition of data. Chao Zhang and Chuanbao Zhang participated in management and analysis of data. Chao Zhang designed the study, and drafted the manuscript. Jing Wang and Weiyan Zhou participated in the revision of the manuscript.

DATA AVAILABILITY STATEMENT

Not applicable.

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REFERENCES

1. Hooi JKY, Lai WY, Ng WK, et al. Global prevalence of Helicobacter pylori infection: systematic review and meta-analysis. *Gastroenterology*. 2017;153(2):420-429.
2. Denic M, Touati E, De Reuse H. Review: pathogenesis of Helicobacter pylori infection. *Helicobacter*. 2020;25(Suppl 1):e12736.
3. Mezmale L, Coelho LG, Bordin D, Leja M. Review: epidemiology of Helicobacter pylori. *Helicobacter*. 2020;25(Suppl 1):e12734.
4. Seo JH, Bortolin K, Jones NL. Review: Helicobacter pylori infection in children. *Helicobacter*. 2020;25(Suppl 1):e12742.
5. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. *CA Cancer J Clin*. 2016;66(2):115-132.
6. Siegel RL, Miller KD, Jemal A, Cancer statistics, 2019. *CA Cancer J Clin*. 2019;69(1):7-34.
7. Strong VE, Wu AW, Selby LV, et al. Differences in gastric cancer survival between the U.S. and China. *J Surg Oncol*. 2015;112(1):31-37.
8. Zong L, Abe M, Seto Y, Ji J. The challenge of screening for early gastric cancer in China. *Lancet*. 2016;388(10060):2606.
9. Gao K, Wu J. National trend of gastric cancer mortality in China (2003–2015): a population-based study. *Cancer Commun*. 2019;39(1):24.
10. Wang FH, Shen L, Li J, et al. The Chinese Society of Clinical Oncology (CSCO): clinical guidelines for the diagnosis and treatment of gastric cancer. *Cancer Commun*. 2019;39(1):10.
11. Mentis A, Lehours P, Megraud F. Epidemiology and diagnosis of Helicobacter pylori infection. *Helicobacter*. 2015;20(Suppl 1):1-7.
12. Eusebi LH, Zagari RM, Bazzoli F. Epidemiology of Helicobacter pylori infection. *Helicobacter*. 2014;19(Suppl 1):1-5.
13. Bessede E, Arantes V, Megraud F, Coelho LG. Diagnosis of Helicobacter pylori infection. *Helicobacter*. 2017;22(Suppl 1):e12404.
14. Sun Y, Zhang J. Helicobacter pylori recrudescence and its influencing factors. *J Cell Mol Med*. 2019;23(12):7919-7925.
15. Fischbach W, Malfertheiner P. Helicobacter pylori infection. *Dtsch Arztebl Int*. 2018;115(25):429-436.
16. Wang K-J, Wang R-T. Meta-analysis on the epidemiology of Helicobacter pylori infection in China. *Zhonghua Liu Xing Bing Xue Za Zhi*. 2003;24(6):443-446.
17. Shi R, Xu S, Zhang H, et al. Prevalence and risk factors for Helicobacter pylori infection in Chinese populations. *Helicobacter*. 2008;13(2):157-165.
18. Zhang W, Hu F, Xiao S, Xu Z. Prevalance of Helicobacter pylori infection in China. *Mod Dig Interv*. 2010;15(5):265-270.

SUPPORTING INFORMATION

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