Letter to the Editor

Evaluation of Colloidal Gold Immunochromatography for the Diagnosis of Human Brucellosis Caused by Smooth *Brucella*[*]

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Brucellosis is an infectious allergic zoonotic disease caused by bacterial species in the genus *Brucella*[1]. It is a Class B infectious disease according to the Law on the Prevention and Control of Infectious Diseases in China. According to the World Health Organization, brucellosis is currently a highly neglected zoonotic disease[2]. Despite this, more than 170 countries have reported cases of brucellosis, and approximately 500,000 new cases are reported each year[3].

Early and accurate detection is the key to preventing, treating, and controlling brucellosis. Currently, the isolation and culture of brucellosis-causing bacteria is the gold standard for diagnosis[4]. However, the clinical isolation rate is low, and the process of culturing is time consuming (often taking up to 4 weeks). The serological diagnosis of brucellosis is generally easier and faster than bacterial isolation and culture, so serological diagnostic methods are widely used. The Rose Bengal plate agglutination test (RBT) is used as a screening test, but its efficiency is affected substantially by the test conditions. Therefore, in China, the serum agglutination test (SAT) is used as a confirmatory test, along with RBT, for the diagnosis of brucellosis[4]. However, the procedures for these tests are complex and time consuming, and the interpretation of the results is easily affected by subjective factors, with false negatives occasionally occurring due to the prozone phenomenon[5]. More reliable tests for the diagnosis of brucellosis are therefore clearly needed.

The colloidal gold immunochromatographic assay (GICA) is a unique immunoassay technique that was developed in the early 1980s. Serological detection with the GICA was added to the Diagnostic Criteria for Brucellosis WS269-2019[4]. This method is easy to perform, and the results are not affected by temperature or time[6]. In addition, it is portable and can be used for the diagnosis and screening of brucellosis in any location, and is especially suitable in emergency situations[6]. Therefore, the application prospects of the GICA are broad. However, because the GICA was only recently added to the Diagnostic Criteria for Brucellosis WS269-2019[4], it is rarely used in hospitals. Therefore, this research seeks to broaden its application by evaluating the validity and reliability of the GICA for brucellosis, using the current standard tests as a reference. We believe that the findings will provide a basis for promoting the development of brucellosis detection technology in China.

This study included 660 patients with suspected brucellosis, who presented with clinical symptoms typical of the disease [fever (≥ 37.5 °C), fatigue, night sweats, and joint pain] and epidemiological risk factors. These patients were admitted to Wulanchabu City Center for Endemic Disease Prevention and Control between March 18, 2019, and December 18, 2019. Their clinical data were recorded, and fasting venous blood (4 mL) was collected for laboratory diagnosis of brucellosis according to the Diagnostic Criteria for Brucellosis WS269-2019[4]. Cases with an antibody titer of ≥ 1:100 (+ +) in the SAT were defined as suspected cases.

SAT antigen was provided by the National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention. The test was conducted according to the Diagnostic Criteria for Brucellosis WS269-2019[4].

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Brucellosis was confirmed if a patient had the clinical symptoms of and the epidemiologic risk factors for brucellosis, and an antibody titer of 1:100 or higher, as measured by the SAT.

A commercial smooth *Brucella* GICA kit was used to test all serum samples. The present GICA manual for testing brucellosis states that both whole blood and serum samples can be used for qualitative detection, but the Diagnostic Criteria for Brucellosis WS269-2019 in China only refers to serum detection[4]. To determine the sensitivity and specificity of GICA detection with whole blood samples and the consistency rate with the SAT, whole blood analysis with the GICA was performed on the first 424 patients and 80 controls during study period. Additionally, 148 positive samples and 30 negative samples were randomly selected to evaluate the GICA kits manufactured by four different companies in China. The sensitivity, specificity, consistency rate, and kappa value obtained using each of the GICA kits were calculated. To evaluate the validity and reliability of the four different GICA kits, the procedures were conducted strictly in accordance with the instructions accompanying each kit. The result was considered to be negative if only the control line appeared red, while it was considered to be positive if both the control line and the test line appeared red.

Microsoft Excel 2010 was used for data analyses. The SAT was used as the confirmatory test. The GICA was compared with the SAT in terms of its sensitivity, specificity, consistency rate, and kappa value. A kappa value of ≤ 0.4 was considered to indicate poor consistency, 0.4 < kappa < 0.75 indicated medium or high consistency, and kappa ≥ 0.75 indicated excellent consistency.

Of the 660 cases of suspected brucellosis, the result of the SAT was positive in 580 cases, including 391 males and 189 females. Their ages ranged between 40 and 65 years, and the average age was 55 years. Of the 580 patients who tested positive, 575 (98.82%) were farmers. With regard to disease severity, 49.31% (286/580) had acute brucellosis (< 3 months), 50.35% (292/580) had chronic brucellosis (> 6 months), and 0.34% (2/580) had subacute brucellosis (3–6 months).

Of the 580 serum samples that tested positive for brucellosis according to the SAT results, the GICA results were positive for serum samples from 574 patients and negative for the 6 remaining serum samples. This indicated a sensitivity of 98.97% (574/580). Of the 80 serum samples that were negative according to the SAT results, 76 were negative and 4 were positive according to the GICA results. This indicated that the specificity of the GICA was 95.00% (76/80) for the serum samples. The consistency rate was 98.48% (650/660), and the kappa value was 0.94 (Table 1).

Among the 424 whole blood samples that tested positive in the SAT, 420 were positive and 4 were negative according to the GICA results. This indicated a sensitivity of 99.06% (420/424). Among the 80 whole blood samples that tested negative in the SAT, 76 were negative and 4 were positive according to the GICA results. This indicated a specificity of 95.00% (76/80). Additionally, the consistency rate was 98.41% (496/504), and the kappa value was 0.94 (Table 2).

Of the 424 positive and 80 negative homologous serum and whole blood samples, as determined by the SAT, 421 homologous serum and whole blood samples were positive, three serum samples were negative, and the three corresponding homologous whole blood samples were positive according to the GICA results. Furthermore, of the 78 homologous serum and whole blood samples that were negative by the SAT, two whole blood samples were negative and the two corresponding homologous serum samples were positive according to the GICA results. The consistency rate was 99.01% (499/504), and the kappa value was 0.96 (Table 3).

In this study, we evaluated the validity and reliability of the GICA antibody detection test for the diagnosis of human brucellosis in China. The

Table 1. Summary of the results of serum analysis by the GICA

GICA (serum)	SAT		Total	Sensitivity (%)	Specificity (%)	Consistency rate (%)	Kappa
	+	−					
+	574	4	578	98.97	95.00	98.48	0.94
−	6	76	82				
Total	580	80	660				

Note. GICA, Colloidal gold immunochromatography; SAT, serum agglutination test.

detection results of the GICA were compared with those of the SAT to determine the sensitivity and specificity of the GICA and its consistency with the SAT. In addition, the GICA was used to analyze whole blood samples for the first time to explore the possibility of introducing whole blood sample testing with the GICA into the standard Diagnostic Criteria for Brucellosis.

The sensitivity and specificity of serum analysis with the GICA were found to be high compared with the SAT. Additionally, the SAT and GICA results showed high consistency. This confirmed that the GICA is an ideal primary screening test for brucellosis. Similar results have been reported by other researchers in China, as well as other countries[7-8]. However, by contrast, Ta[6] tested 1,088 serum samples from suspected brucellosis cases in eight cities in Inner Mongolia Autonomous Region, China, and found that the sensitivity of the GICA was only 74.8%.

To date, all of the articles published in China about brucellosis detection with the GICA have used serum samples only[9], and no previous reports have analyzed whole blood samples with the GICA. This study is the first to use the GICA for the analysis of whole blood samples and to report its sensitivity, specificity, and consistency with the SAT for brucellosis detection in whole blood samples. The results showed that the GICA had 99.06% sensitivity and 95.00% specificity, with a consistency rate of 98.41% with the SAT (kappa = 0.94) for brucellosis detection in whole blood samples. In addition, the GICA was used to analyze 504 homologous serum and whole blood samples, and the results of whole blood analysis with the GICA were highly consistent with those of homologous serum analysis with the SAT and serum analysis with the GICA. However, according to the Diagnostic Criteria for Brucellosis WS269-2019[4], only serum samples can be used for the GICA. Based on the present findings, obtained under strict quality control conditions, it is recommended that GICA-based detection of brucellosis in whole blood samples be added to the Diagnostic Criteria for Brucellosis in China. This would be beneficial for the faster and easier detection of brucellosis, as well as its timely diagnosis and treatment. The detection of brucellosis in whole blood samples by the GICA requires only a small volume of whole blood, and the procedure is more convenient than serum analysis. The findings can be interpreted in an intuitive and simple way that does not require special instruments or equipment, professional laboratories, or professional training. Furthermore, even grass-root units in remote areas can carry out such tests, and the methodology is especially suitable for dealing with emergencies[6].

In summary, the detection of brucellosis in serum or whole blood samples by the GICA has several advantages, such as its high sensitivity and specificity, the speed and simplicity of the assay, and the ease of preservation of reagents. Therefore, it is an ideal primary screening test for brucellosis, especially for the on-site analysis of samples and

Table 2. Summary of the results of whole blood analysis by the GICA

GICA (whole blood)	SAT		Total	Sensitivity (%)	Specificity (%)	Consistency rate (%)	Kappa
	+	−					
+	420	4	424	99.06	95.00	98.41	0.94
−	4	76	80				
Total	424	80	504				

Note. GICA, Colloidal gold immunochromatography; SAT, serum agglutination test.

Table 3. Summary of the results of GICA analysis of homologous serum and whole blood samples

GICA (whole blood)	GICA (serum)		Total	Consistency rate (%)	Kappa
	+	−			
+	421	3	424	99.01	0.96
−	2	78	80		
Total	423	81	504		

Note. GICA, Colloidal gold immunochromatography.

large-scale sample testing. Given these advantages, the assay has broad application prospects, especially in remote and rural areas[10]. In the future, the reproducibility of whole blood sample analysis with the GICA should be determined, so that it can be used for screening brucellosis in China on a larger scale.

Ethical Approval We confirmed that the identification information for all participants (including patient names, ID numbers, home addresses, and telephone numbers) would not be included in recordings, written descriptions, or publications. The blood samples used in this study were all taken after hospital diagnosis, and there were no ethical issues involved.

Conflicts of Interest The authors declare no conflicts of interest.

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