COMPARATIVE STUDY ON RAPID DOT-IMMUNOGOLD STAINING AND TWO IMMUNOGOLD SILVER STAINING ASSAYS FOR DIAGNOSING SCHISTOSOMIASIS JAPONICA

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Abstract. A fast, specific, sensitive, convenient, and economical rapid-dot-immunogold staining (R-Dot-IGS) assay was used to detect serum antibodies in patients infected with *Schistosoma japonicum.* The soluble egg antigen of *Schistosoma japonicum* was added onto microspore membrane. After pre-reacting and blocking, the serum to be detected and sheep anti-human IgG labeled with chloroauric acid were added sequentially. The assay took 15 minutes. For comparison, the dot-immunogold silver staining (Dot-IGSS) and rapid micro-volume Dot-IGSS (RM-Dot-IGSS) assay were also performed. The positive rate to detect the serum of schistosomiasis japonica by the R-Dot-IGS, Dot-IGSS and RM-Dot-IGSS assay was 98%, 98% and 100%, respectively. Samples from 50 healthy controls, 10 cases of clonorchiasis, and 10 cases of paragonimiasis showed negative reactions except for one case of clonorchiasis with RM-Dot-IGSS assay. Compared with Dot-IGSS and RM-Dot-IGSS, R-Dot-IGS assay has similar sensitivity and specificity, but the latter is quicker, simpler, and cheaper. Therefore, R-Dot-IGS is strongly recommended for rapid diagnosis of schistosomiasis japonica both in epidemiological study and in the clinic.

Schistosomiasis japonica is one of the parasitic diseases that severely damages human health. This disease was under control in some southern area of China. Even with long period and large scope of prevention and treatment, acute and repeated infections often happen in some epidemic area of China every year. One important problem is how to make an accurate diagnosis for schistosomiasis japonica on time, especially in slight and chronic infection patients. The accuracy of parasitic examination should be pursued. Developing a rapid, simple, sensitive, specific and convenient immmunological assay is a crucial step. Dot-immunogold silver staining (Dot-IGSS) is a highly sensitive and specific assay when it is used to detect anti-parasite antibodies (Wu *et al*, 1989; Liu *et al*, 1996, 2001), especially in the diagnosis of schistosomiasis japonica (Zheng *et al*, 1994). We have established a rapid micro-volume Dot-IGSS (RM-Dot-IGSS) to detect the serum antibodies of the patients with cysticercosis (Fu *et al*, 2000). The total time of this measurement is less than 30 minutes. By further modifying the assay, we established rapid-dot-immunogold staining (R-Dot-IGS). We applied R-Dot-IGS to cysticercosis and achieved good results (Zheng *et al*, 2002). In order to determine whether the assay is suitable for clinical diagnosis and epidemiological survey of schistosomiasis japonica, we compared R-Dot-IGS with Dot-IGSS and RM-Dot-IGSS to detect anti-*Schistosoma* antibodies in sera from patients infected with *Schistosoma japonicum.*

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MATERIALS AND METHODS

Materials and reagent for R-Dot-IGS

Vehicle. Mixed cellulose ester microspore filter membrane (MCE) was used as vehicle, the pore diameter of which was 0.45 μm.

Antigen. The soluble egg antigen of *Schistosoma japonicum* (ESA) was provided by Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, with nitrogen concentration

being 3 mg/ml. It was diluted to 150 µg/ml before use.

Serum. Sera from 55 schistosomiasis japonica patients with stool positive for *Schistosoma japonicum* were provided by Institute of Parasitic Diseases of Jiangxi Province. Control sera were from 50 blood donors provided by Xuzhou Red Cross Blood Service, Jiangsu Province, 10 cases of clonorchiasis patients living in Pizhou city, Jiangsu Province, and 10 cases of paragonimiasis provided by Institute of Parasitic diseases, Zejiang Academy of Medicine.

Sheep anti-human IgG labeled with chloroauric acid (GSAHIgG). The sheep anti-human IgG was provided by SIND-American Biotechnology Co. The colloidal gold was 5 nm in diameter. The sheep anti-human IgG was labeled in our laboratory according to Slot's method (Slot and Genuze, 1985) and kept preserved at -20ºC.

Blocking solution. The solution was 0.02 mol/l TBS pH 8.2 containing 1% bovine serum albumin (BSA).

R-Dot-IGS procedure

Antigen addition. The MCE paper was divided by pencil into 1 mm squares. A drop of the ESA (about 1.5 µl) was spotted with a pin onto the center of each small square and the paper was dried at room temperature (RT) and stored at 4ºC.

Pre-reaction. The paper was taken from the refrigerator and was immersed in 0.02 mol/l TBS pH 8.2 for 1 minute at room temperature (RT). After that, the paper was removed from TBS and the liquid on the membrane was absorbed with filter paper.

Blocking. The membrane was placed upon a plastic plate, absorbed with filter paper again, then blocking solution was spotted onto the center of the square, 10 µl per square. The plate was incubated at RT for 3 minutes.

Sera addition. The sera being detected were spotted onto the center of the squares, 2 µl per square, and the plate was incubated at RT for 3 minutes.

GSAHIgG addition. Ten µl of GSAHIgG were added to each square and the plate was incubated at RT for 4 minutes, then the plate was washed with tap water and absorbed with filter paper.

Fig 1–The positive and negative results of R-Dot-IGS.

Results readout. Positive results were decided by the appearance of regular, round, brown-red dot at the center of the square of MCE. The negative result was that no dot appeared on the MEC and only irregular, light brown trace appeared (Fig 1).

Dot-IGSS and RM-Dot-IGSS procedure

Dot-IGSS and RM-Dot-IGSS were performed according to methods previously reported (Fu *et al*, 2000, Liu *et al*, 1994, 1997). In these assays, some procedure were modified to obtain optimum conditions. The main reagents and basic procedure of the three assays are shown in Table 1.

RESULTS

Positive rate of the three assays

The results of R-Dot-IGS, RM Dot-IGSS and Dot-IGSS assays for detecting anti-*Schistosoma japonicum* antibodies in different sera are shown in Table 2. There was no significant difference in the positive rate among the three assays ($p>0.05$).

Dot intensity of the three assays

The intensity of the dot color was arbitrarily judged with naked eye as 1+, 2+, 3+ and 4+. The results from of R-Dot-IGS and RM-Dot-IGSS assay are shown in Table 3.

Among the 55 serum specimens, 20 had the same dot reaction intensity in R-Dot-IGS and RM-Dot-IGSS, 20 had higher intensity in R-Dot-IGS than in RM-Dot-IGSS and 15 lower intensity in the former than in the latter assay. There is no significant difference in dot reaction intensity between the two rapid assays ($u=0.275$, $p > 0.05$).

Table 1
Comparison of main reagents and basic procedure of the three assays.

Item	R-Dot-IGS	RM-Dot-IGSS	Dot-IGSS
Vehicle	Microspore filter membrane	Microspore filter membrane	Microspore filter membrane
Concentration of ESA	150 μg/ml	150 μg/ml	150 μg/ml
Concentration of sera	No diluting	No diluting	1:20
Concentration of GSAHIgG	No diluting	1:10	1:30
Blocking solution	1%BSA	1%BSA+10% sheep serum	1%BSA+10% sheep serum
Deluding solution	-	1%BSA+10% sheep serum	10% sheep serum
Pre-reacting	1 minute	1 minute	-
The first blocking	-	3 minutes	10 minutes
Reaction after adding serum	3-5 minutes at RT	3-5 minutes at RT	120 minutes, 37ºC
Washing	-	-	5 minutes, 3 times
The second blocking	-	-	10 minutes
Reaction after adding GSAHIgG	3-5 minutes, 25ºC	3-5 minutes, 25ºC	60 minutes, 37ºC
Washing	Washing, drying, judging results	3 times, total 1 minute	5 minutes, 3 times
Condition of developing	-	Darkroom	Darkroom
Developing	-	3-5 minutes at RT	3-5 minutes, 30º-37ºC
Washing	-	3 times, total 1 minute	3 times, total 1 minute
Total time	<20 minutes	About 30 minutes	About 5 hours

Table 2
Results of anti-*Schistosoma japonicum* antibody detected by R-Dot-IGS, RM-Dot-IGSS and Dot-IGSS.

Serum	No. cases	No. of positive (%)		
		R-Dot-IGS	RM-Dot-IGSS	Dot-IGSS
Schistosomiasis japonica	55	54 (98)	54 (98)	55 (100)
Donor	50	0 (0)	0 (0)	0 (0)
Clonorchiasis	10	0 (0)	1 (90)	0 (0)
Paragonimiasis	10	0 (0)	0 (0)	0 (0)

DISCUSSION

When immunogold silver staining (Dot-IGSS) to detect the antibodies from patients infected with *Clonorchis sinensis* was established in 1989 as a new immunological diagnosis method (Wu *et al*, 1989), which was sensitive, specific, and easy to observe results, Dot-IGSS has been used widely for the immunological diagnosis of parasitic diseases. After that, human sera antibodies against *Wuchereria bancrofti* and cysticercosis have been detected with Dot-IGSS or modified Dot-IGSS such as RM-Dot-IGSS in our laboratory (Liu *et al*, 1994,

Table 3
Reaction intensity of sera of patients with schistosomiasis japonica detected by R-Dot-IGS and RM Dot-IGSS.

Reaction intensity	Number	
	R-Dot-IGS	RM-Dot-IGSS
-	1	1
1+	2	10
2+	22	14
3+	24	21
4+	6	9
Total	55	55

1997; Fu *et al*, 2000). When sera from patients with cysticercosis or schistosomiasis were detected by RM-Dot-IGSS, sensitivity and specificity were very high (Wu *et al*, 1993; Fu *et al*, 2000). On the other hand, results readout from these assays needs developing of silver nitrate in a darkroom. It is difficult for nonpractised experimenter to estimate the developing time.

Based on Dot-IGSS and RM-Dot-IGSS assays, experimental conditions were modified with the elimination of silver nitrate. Positive colloidal gold color appeared on the filter membrane directly, and R-Dot-IGS was thus established. Not only was the assay entirely conducted in the light and at room temperature, but also the time of experiment was shortened significantly. If the antigen was dropped on the membrane beforehand and stored in refrigerator, it took only 15 minutes from the time sera were dropped for the results to come out. Even with 30 specimens the experiment can be done in 25 minutes. The results of R-Dot-IGS were also preserved for a long time with persistent dot color. In addition, the R-Dot-IGS can be done using simple equipment. The membrane was directly set on a plastic plate that can be re-used many times. The numbers of the small squares of membrane may be cut out from MCE according to the quantity of the specimen so it is ideally suited to detect one serum or many serum specimens.

Our results showed that the sensitivity and specificity of R-Dot-IGS was 98% and 100% respectively when it was used to detect the sera anti-schitosoma antibodies. We found no cross reaction with sera from patients infected with *Paragonimus westermani* or *Clonorchis sinensis* and no false positive reaction from healthy human samples. Compared with Dot-IGSS and RM-Dot-IGSS, R-Dot-IGS had similar results, but with much shorter time (one-twentieth of Dot-IGSS and one half of RM-Dot-IGSS). We consider that R-Dot-IGS assay is not only very fast and simple, but also is very stable, sensitive, specific, and practical. It can be used to quickly diagnose schistosomiasis japonica both in field situation and in the clinic.

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