Development and application of diagnostics in the national schistosomiasis control programme in the People’s Republic of China

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Abstract
Schistosomiasis, caused by Schistosoma japonicum, has a documented history of more than 2,100 years in the People’s Republic of China (P.R. China). In spite of great progress in controlling the disease, it is still one of the most serious parasitic diseases in the country. The study and use of diagnostic techniques play an important role in the targeting of chemotherapy that has been continuously applied in the national schistosomiasis control programme for 60 years. This paper reviews the development and application of parasitological methods, immunodiagnostic and molecular diagnostic technology for S. japonicum with a brief mention of imaging diagnosis. When analysing the efficacy and performance characteristics of the main diagnostic techniques in current use, it becomes apparent that approaches that worked well in the past are less suitable now when successful control is shifting the endemic situation towards transmission control and elimination of schistosomiasis as a public health problem. The conclusion is that a mutable approach must be adopted choosing the most appropriate diagnostic technique for each control stage and area, thus modifying the methodology according to the prevailing need in terms of sensitivity and specificity.

1. Introduction
Schistosomiasis in the People’s Republic of China (P. R. China) is confined to infection by Schistosoma japonicum. The first report of a case where the clinical symptoms were directly connected with this parasite was made by an American physician working in P. R. China (Logan, 1905). After that, only sporadic reports are available in English, but in the mid-1950s, there were more than 11 million known infected cases (Chen and Feng, 1999, Mao and Shao, 1982a). Thanks to sustained,
strong control efforts, both prevalence rates and the intensity of infection in the endemic areas have dramatically declined since then resulting in a drop to an estimated 185,000 people in 2013 (Lei et al., 2014, Wang, 2006). According to the criteria for schistosomiasis control and prevention issued by Chinese government, the stages for schistosomiasis control of the Chinese national control programme are classified as morbidity control (prevalence >5%), infection control (prevalence = 1-5%), transmission control (prevalence lower than 1%), transmission interruption (no case detected locally for five years) and elimination (no case detected locally for five years continuously after transmission was interrupted) (Zhou et al., 2011). Case finding and treatment, assessment of morbidity as well as evaluation of control strategies all build on the results of diagnostic tests, so it can emphatically be said that diagnosis is the essential basis of treatment and central to the control of schistosomiasis (Feldmeier and Poggensee, 1993).

The current widely used methodology for schistosomiasis japonica diagnosis can be divided into parasitological techniques (detection of parasite eggs or miracidium in urine or faeces) and immunologic approaches (detection of antibody or circulating antigens). The direct parasitological techniques such as Kato-Katz thick smear method, miracidium hatching technique are used to determine the infection and usually serve as a gold standard or reference method to evaluate other diagnostic tools (Bergquist et al., 2009, Wu, 2002a). Indirect approaches including immunodiagnostic techniques have superior sensitivity and are rapid and affordable. Antibody-based immunoassays are useful for large-scale screening but positive titres abate only slowly making it impossible to differentiate between current and cured infections. On the other hand, antibody techniques are highly effective for surveying areas from where infection has been eliminated (Hillyer et al., 1999). The detection of circulating antigens is not only the most reliable technique, but also the most direct one as the amount of released antigens from the adult worms in the host only change with a change in the worm burden, while the excretion of eggs from the infected host is uneven, in particular in the case of S. japonicum. In addition, the development of molecular biotechnology, including the polymerase chain reaction (PCR) and the loop-mediated isothermal amplification (LAMP), both based on the gene cloning amplification, have been developed and successfully transferred from the laboratory to the field (Lier et al., 2006). To provide guidance and standardize the diagnostic work for disease control staff and clinical doctors, diagnostic criteria for schistosomiasis was issued and updated by ministry of health according to the development of diagnostic techniques and clinical symptoms of schistosomiasis japonica (Ref).

Integration of diagnostic tools into national control programmes requires collaboration between research laboratories, epidemiologists and control programme managers, defining assays in terms of sensitivity, specificity and predictive values. During the process of schistosomiasis control in P.R. China, tools used for clinical diagnosis, monitoring control effect, epidemiological survey, targeting chemotherapy
etc. are shift with the development of drugs for treatment, diagnostic techniques as well as the decrease of prevalence and infection intensity of schistosomiasis. P.R. China has now reached the stage when priority is shifting from traditional monitoring and control to surveillance and response with the focus on re-infection in areas targeted for elimination. The most urgent need at this time is reliable assessment of control efficacy and the determination of target populations for chemotherapy in different areas and at endemic stages using sensitive and specific assays. This review discusses the currently most important diagnostic approaches useful for epidemiological survey against the backdrop of the historical record.

2. Historical review of diagnostic tools for schistosomiasis developed or used in P.R. China

2.1 Parasitological methods
The diagnosis of active *S. japonicum* infection consists of the detection of viable schistosome eggs in faeces or tissue biopsies. Although of little importance in the endemic areas, it should be pointed out that acute schistosomiasis, particularly in the very early stages and in ‘immunologically naive’ patients, could sometimes remain undiagnosed by this approach alone. Proctoscopy combined with rectal biopsy is a clinical, hospital-based approach that does not play a role in large-scale control schemes. In addition, poor acceptance by patients and physicians alike, due to the associated complications, such as bleeding and high possibility of sampling error on collection, limits the use of this approach in practice.

For quantification of faecal egg counts, the Kato–Katz method, originally developed in the mid-1950s by the Japanese researchers Kato and Miura (Kato and Miura, 1954) and later standardized by the introduction of a 41.7 mg templates by Katz and colleagues in Brazil (Katz et al., 1972), is the most broadly used technique in epidemiological surveys pertaining to intestinal schistosomiasis. The number of eggs per gram faeces is used as an index of the intensity of infection and cases could be divided to three categories with light infection (EPG<100), medium infection (100<EPG<800) and heavy infection (EPG>800) according to the criteria of WHO. In spite of reduced utility with regard to light infections, the Kato–Katz thick stool smear remains recommended by the World Health Organization (WHO) for prevalence mapping and field-based control of schistosomiasis. Among traditional tests, the hatching test, based on the positive phototrophic behavior of schistosome miracidia, relies on the observation of released miracidia from parasite eggs hatched in faeces diluted in water and allowed to sediment (Qiu and Xue, 1990). The sensitivity of hatching technique is supposed higher than that of Kato-Katz thick smear method due to the large volume of faeces detected (Ref). Sometimes, hatching technique followed by microscopic examination for the fecal sediment are implemented to improve sensitivity (Ref). However, it is influenced by temperature, quality of the hatching
water and examiner experience. The slight improvement of sensitivity achieved may not justify wider use due to the added time and cost required. Both techniques are simple but require microscope, well-trained laboratory technicians and mostly reusable test kit materials.

2.2 Imaging techniques

Ultrasonography originated in 1940s (Zhou et al., 2009), but has not been used for schistosomiasis investigations until the 1980s. Although radiology, now generally replaced by computed tomography (CT) and magnetic resonance imaging (MRI) for space-occupying lesions or schistosome-induced hepatic disease, must be carried out in a hospital environment, ultrasound (US) has several advantages. Not only can the technology be adopted for field use, but it is also less costly while still capable of demonstrating the classical features of periportal fibrosis (appearing as a netlike echogenic pattern in *S. japonicum* infection), hepatic granuloma and gallbladder thickening. Especially for advanced schistosomiasis, US results are used not only in the diagnosis and differential diagnosis of advanced schistosomiasis, but also for the guidance of treatment and evaluation of therapeutic effects and, furthermore, for risk predictions of portal hypertension and upper gastrointestinal haemorrhage (Wu et al., 2015). Indeed, the use of portable US equipment has broadened the applicability of diagnostic, imagery investigations in endemic community settings. The diagnostic characteristics of all three methodologies are, however, similar in that they are non-invasive, simple and record the damage of intra-abdominal organs in a straightforward way. Imaging approaches are also suitable for diagnosis of ectopic schistosomiasis, e.g. neuroschistosomiasis (Ross et al., 2012). Although CT and MRI are not routinely used for schistosomiasis diagnosis in resource-poor areas for economic reasons, all three techniques require highly qualified users with medical education and special training. Together with CT and MRI, US facilitates the detection of lesions, such as pathological enlargements and tissue oedema, which is suitable for location before clinical, invasive procedures (Fang and Xu, 2014, Li, 2015, Lv and Pei, 2007, Shen et al., 2012, Wang et al., 2014, Yang, 2015, Zhang et al., 2012b).

2.3 Immunological tests

Immunological tests have a long history in P.R. China for schistosomiasis diagnosis due to advantages such as high sensitivity, easy use and rapidity. These tests are developed to detect the circulating antibody or antigens of schistosomes based on various labelling techniques.

Intradermal tests (ID) were the first successfully developed immunodiagnostic method. The strategy of screening a population by ID first followed by stool examination only for the ID positives made large-scale screening feasible and allowed understand the distribution and approximate prevalence of schistosomiasis in a short time during the 1950s (Maegraith, 1958, Mao and Shao, 1982b).

2.3.1 Intradermal test (ID)

The earliest antibody test in P.R. China was the intradermal test (ID) introduced by Gan (Gan, 1936), who adopted this approach from work described for other
schistosome species. Antigens used for ID to detect specific antibody IgE were extracted from fresh or frozen adult worms, eggs, miracidia or cercaria and their diagnostic efficacy was evaluated. The advantages of ID such as ease of use, low cost and high sensitivity of 90% facilitated its implementation in the early 1950s in the national schistosomiasis control programme to investigate the distribution and prevalence of \textit{S. japonicum}. But it was soon replaced by other tests.

2.3.2 Circumoval precipitin test
The circumoval precipitin test (COPT) was originally described by Liu et al. (Liu et al., 1958) and widely used in P.R. China in the remaining part of last century. COPT substitutes intact fresh parasite eggs for the antigen preparation, normally consisting of eggs that are lyophilized or subjected to heat and ultrasound. It has a high sensitivity (94–99%) and adequate specificity with low false positive rates in healthy people from a non-endemic areas (2–4%). After effective treatment for 4 or 3–8 years, the negative reversion rates were 82.5% and 80–83%, respectively (Li, 1991). However, with repeated treatments in the controlled areas leading to very low rates of prevalence and intensity of infection, the sensitivity of the test declined to 70–80% (Song et al., 2003). The technique is also comparatively complicated and time-consuming (48 hours for recording the results in the laboratory) and requires microscopy, issues that limit a wider application in endemic areas these days.

2.3.3 Indirect hemagglutination assay
Soluble antigen preparations are necessary for tests relying on agglutination of microscopic particles, e.g. sheep erythrocytes as used in the indirect hemagglutination assay (IHA), first employed for the diagnosis of \textit{S. japonicum} in P.R. China by Tao (Shi et al., 1980). IHA remains a widely used general immunoassay in P.R. China, secondary only to COPT in having been used for more than 50 years(Zhu et al., 2009). The accuracy of the test is entirely dependent on the quality of the antigen used to coat the red blood cells. With a highly purified antigen preparation, IHA has been shown to reach 93-100% sensitivity, while the false positive rate in healthy people from non-endemic areas can be reduced to 2-3%. For example, the cross-reaction with \textit{Paragonimus westermani} is 64-84% with the crude soluble egg antigen (SEA) and still 31% with the purified egg antigen (Wu et al., 1991). Nevertheless, after effective, periodical treatment for 3 years or more, most former schistosomiasis patients turned negative in the test. IHA continues to be applied for community diagnosis and screening of people targeted for chemotherapy and has been used for immunodiagnosis in 80 nationwide schistosomiasis surveillance sites since 2005 (Zhu et al., 2009).

2.3.4 Enzyme-linked immunosorbent assay
Yan and Lv (Yan and Lv, 1978) were the first to develop and use the enzyme-linked immunosorbent assay (ELISA) for the diagnosis of schistosomiasis in P.R. China, a reliable, sensitive and specific diagnostic tool that is now regarded as meeting all stringent requirements for field use. The sensitivity of routine applications
of ELISA based on SEA for schistosomiasis reaches 95 to 99% in *S. japonicum* egg-positive individuals, while the false positive rate in a non-endemic area is 1-4% (Li et al.?). The serologic reversal rate 1-2 years after effective treatment was 59-60% (Li, 1991). However, ELISA is mainly a laboratory-based tool useful for large-scale operations with field application attached with considerable difficulty as an ELISA reader is required and the delay before it is possible to inspect the results is usually 2-3 hours. However special treatment of the antigens can extend its application. For example, the Falcon assay screening test (FAST)-ELISA with a 1% mixture of SEA and urea soluble antigen (mixed with the same amount of SEA and urea-soluble egg) as the detecting antigen can achieve the same sensitivity and specificity as routine SEA-ELISA (Wang and Li, 1990). Indeed, ELISA is a versatile test and appeared soon in various modifications apart from FAST-ELISA, such as ABC-ELISA (Zhang et al., 1986), dot-ELISA and the fractioned antigen (FA)-ELISA (Zhu et al., 1996).

The avidin-biotin-peroxidase complex enzyme-linked immunosorbent assay (ABC-ELISA) is an amplification based on a monoclonal antibody against the *S. japonicum* gut-associated circulating antigen (GAA) (Chen et al., 2006). The test has a sensitivity of 83.1% and a specificity of 94.0% in previously infected persons, while it is as high as 94.0% in acute schistosomiasis. The rate of cross-reaction with antigens of *Paragonimus*, *Conorchis* and Hepatitis B virus was 12.9%, 15.8% and 13.0% respectively (also Chen et al., 2006). However, 6 and 12 months after treatment of chronic schistosomiasis, the rates of seroconversion was 43.9% and 62.1%, respectively (also Chen et al., 2006).

The dot-ELISA) is a highly versatile solid-phase immunoassay for antibody or antigen detection. The assay uses minute amounts of reagent dotted onto solid surfaces such as nitrocellulose and other paper membranes which avidly bind proteins. After incubation with antigen-specific antibody and enzyme-conjugated anti-antibody, the addition of a precipitable, chromogenic substrate causes the formation of a coloured dot on the solid phase which is visually read (Pappas, 1988). Using this approach with a monoclonal antibody against the cathodic *S. japonicum* GAA linked with peroxidase to detect the antigen in sera, Yan et al. (Yan et al., 1990) found positive rates of the acute and chronic stages of schistosomiasis patients at 90.6% and 83.2%, respectively. No positive reactions were found with sera from patients infected with *Clonorchis, malaria* and some other non-parasitic diseases. The serum conversion rate was 84.0% of patients with negative faecal examination one year after praziquantel treatment.

The FA-ELISA test, based on the 107–121 kDa fraction of SEA, not only reached a high sensitivity (91–95%) and specificity (97%–100%), similar to routine SEA-ELISA, but also showed a high efficacy with respect to serum conversion after cure with a rate over 90% 18 months after treatment (Zhu et al., 2012). Almost no cross-reaction with was seen with *P. westermani*, *Clonorchis sinensis* and *Fasciola buski*, which makes it a better test than routine SEA-ELISA (Hua et al., 1996, Liang et al., 2001, Zhu et al., 1996).
Another approach improving the scope of ELISA application is the use an anti-idiotype antibody to mimic specific schistosome antigens. For example, a monoclonal antibody mimicking the GAA makes it possible to use the ELISA routine for the diagnosis of acute schistosomiasis (positive rates: 95-100%). However, the positive rate with this modification for chronic cases was only 61-73%, i.e. lower than that of routine SEA-ELISA (Guan et al., 1991, Tao et al., 1996, Wu et al., 1993). However, this approach has a higher efficacy on evaluation of cre after treatment, i.e. the negative conversion rate one year after effective treatment was 43-100% (Huang et al., 1999).

2.3.5 Rapid diagnostic kits
In the late 1990s, the dot immunogold filtration assay (DIGFA), a rapid diagnostic assays based on SEA as antigen and rabbit-anti-human IgG labelled with colloidal gold as probe, was developed (Ding et al., 1998). The sensitivity and specificity of the assay were 98 and 100%, respectively, and apart from P. westermani (6% cross-reaction), there were no cross reactions with several other common parasitic antigens (Ding et al., 1998). Tang et al. (Tang et al., 2008) developed the assay further by using a sheep anti-human IgM immunogold conjugate as probe to detect specific IgM anti-S. japonicum antibody that produced positive rates for acute and chronic schistosomiasis at 100% and 96%, respectively. Only 3% of patients infected with P. westermani were positive. The rates of seroconversion in chronic schistosomiasis at the 6th month and 12th month intervals after treatment were 65% and 88%, respectively.

Another rapid diagnostic assay, the dipstick dye immunoassay (DDIA) (He et al., 2000), is basically a chromatography technique suitable for field application using SEA labelled with a dye as the indicator system,. This approach was further developed by Zhu et al. (Zhu et al., 2005b, Zhu et al., 2002) and Song et al. (Song et al., 2003). The test shows a high sensitivity for both acute and chronic cases of schistosomiasis (97% and 94-97%, respectively) and has 97% specificity when tested with healthy people from non-endemic communities. Apart from P. westermani, cross-reactions with other common parasitic diseases are low (<10%). DDIA is also inexpensive, rapid (5-10 min), simple to perform and does not require special equipment.

Yu and Ding (2010) developed a novel, rapid diagnostic assay called dipstick latex immunochromatography assay (DLIA), which has subsequently been modified for the detection of antibodies in whole-blood samples (WB-DLIA)!!! INVALID CITATION !!!). This test is an indirect immunoassay for detecting anti-S. japonicum antibodies in serum or whole-blood. The dipstick used SEA as the target antigen on the test line with goat anti-mouse IgG as the second antibody on the control line. Red latex particles labelled with mouse anti-human IgG was used as colour probe. The research showed that the DLIA assay has a high sensitivity (95%
for serum and 94% for whole-blood) and specificity (95% for serum and 97% for whole-blood) with no cross-reaction with Clonorchis or intestinal nematodes including Angiostrongylus cantonensis. The Youden index (ref) for DLIA was 0.90 and 0.91 for schistosomiasis diagnosis in serum and whole-blood, respectively. The assay is both practical and simple and since neither microscope, nor special equipment or serum separation are needed it is very suitable for general field screenings.

The assays discussed above are all useful for disease surveillance and for preliminary field screenings in communities with low infection rates at the various of control stages. IHA and ELISA are inexpensive, convenient and semi-quantitative; some tests even be termed quantitative. DIGFA, DDIA and DLIA provide rapid, simple, convenient and reliable diagnosis of schistosomiasis, especially for large-scale screening of patients (Table 1). Compared to stool examination, these serological tests give up to 90% more positive data but, on the other hand, they do not differentiate between current and past infection, which is of particular relevance in areas of low endemicity. In addition, cross-reactions are frequently encountered because of the use of crude parasite antigens material or soluble parasites, e.g. SEA that contains many antigens cross-reacting with antigens from unrelated species. On the other hand, these problems can be solved by adapting the techniques for use with purified antigens.

With the advent of the hybridoma technique by (Kohler and Milstein, 1975), a number of monoclonal antibodies reactive with specific schistosome antigens, were developed in P.R. China and used for the detection of circulating schistosome antigens (Zhu, 2005). In the past twenty years, many assays based on recombinant peptide antigens, such as rSj26GST (Xie et al., 1995), rSj23HD (Ren et al., 2001), rSj32 (Shu et al., 1998) and a monoclonal anti-SjCTPI antibody was developed (Zhu et al., 2000). Recently, the rSP13-ELISA tool developed by Dr. Xu and his colleagues showed substantial advantages over egg-detection and SEA-ELISA, including adequate sensitivity and specificity needed in areas characterized by low levels of transmission (!!! INVALID CITATION !!!). Application of such tools may allow identification of cases with low-intensity infections and targeted treatment (Zhou, 2014). Along with the reduced prevalence, in particular the reduced intensity of infection resulting from the success of the ongoing control programmes, many previously used techniques are not sufficiently sensitive and must therefore be augmented or replaced by immunological techniques based on both antigen and antibody detection (Bergquist et al., 2009, Bergquist et al., 2015, Doenhoff et al., 2004, van Lieshout et al., 2000, Wu, 2002b).

2.4 Techniques based on molecular biotechnology

Better diagnostic tests for schistosomiasis are needed both in the field and in the clinic. Future research for the diagnosis of aetiology may depend on molecular tools such as PCR-based approaches, which have shown great sensitivity and specificity for detection of Schistosoma DNA in a variety of samples (Gomes et al., 2010, Pontes et
Indeed, PCR is one of the most sensitive techniques of analysing DNA and is increasingly used in studies to diagnose schistosomiasis. However, the dependence on an expensive apparatus and the need for specialized, trained technicians restricts their widespread applications for testing under field conditions. (Guo et al., 2012, Kato-Hayashi et al., 2015, Sandoval et al., 2006)

Lier et al. (Lier et al., 2008, Lier et al., 2006, Lier et al., 2009) designed a novel, real-time PCR method targeting the mitochondrial gene encoding NADH dehydrogenase I, a flavoprotein that contains iron-sulfur that can detect *S. japonicum* DNA at the level of one EPG, is also specific for *S. japonicum* when tested against other *Schistosoma* species, *Trichuris trichiura*, hookworm and *Taenia sp*. Xia et al. (Xia et al., 2009) and Fung et al. (Fung et al., 2012) evaluated a PCR assay to detect *S. japonicum* infection in humans and bovines in P.R. China. The test was highly sensitive, detecting *S. japonicum* DNA at 0.5~1.1 eggs/g of stool. *S. japonicum* DNA was detected in sera at the first week post-infection (doesn’t sound right), and became negative 10 weeks post-treatment, whereas the anti-worm IgG gave positive results at 4-6 weeks and still at high levels 23 weeks post-treatment (ref). Comparing PCR examination of a single stool sample to the hatching test using three consecutive stool samples, more humans were positive for the latter (20%) than the PCR test (15%) (ref). However, two individuals were PCR positive in a village where no infections were detected by traditional methods (ref).

The LAMP assay, first reported by Notomi et al. (Notomi et al., 2000), is a highly sensitive test that would be useful for detection of light intensity of infection or false-negative patients and for confirmation of cure. The assay is based on the sequence of a highly repetitive retrotransposon (*SjR2*) and has been shown to detect 0.08 fg *S. japonicum* DNA, which is 10^4 times more sensitive than conventional PCR (Xu et al., 2015, Xu et al., 2010). Unlike PCR, the LAMP assay does not require amplification cycles by thermocycling or amplicon detection by electrophoresis. The test has a high sensitivity of 95~97% for the diagnosis of *S. japonicum*-infected patients with the lowest intensity (EPG<10) (ref) (this is not more sensitive than PCR). For the assessment of efficacy after treatment with praziquantel, the negative conversion rate increased from 23.4%, 61.7% to 83.0% at 3 months, 6 months and 9 months post-treatment by LAMP, whereas for ELISA and IHA, the negative conversion rate remained at a low level (25.5% by ELISA and 31.9% by IHA) even at 9 months after treatment (Xu et al., 2015, Xu et al., 2010). Xu et al. (Guo et al., 2013) has also proved that the LAMP assays have some advantage over PCR. These results demonstrate that PCR assay and LAMP were effective tools for early diagnosis and evaluation of therapy effectiveness for *S. japonicum* infection in schistosomiasis-endemic areas with low-intensity infection classified as transmission-interrupted.

### 3. Diagnostic applications in the national schistosomiasis control programme

#### 3.1 Application of diagnosis for schistosomiasis control in P. R China

Since the 1950s when the national campaign against schistosomiasis began in
China, the application and development of diagnostic techniques have undergone shifts. However, an approach that is useful in practice is almost always a compromise between quality and quantity because the techniques needed for large-scale application must be based on cost-effectiveness, precision, simplicity and stability (Bergquist et al., 2009). The key of the matter is the required continuous adaption of the diagnostic focus to the stage of control. Table 1 shows the various stages of a schistosomiasis-control programme juxtaposed with the type of diagnostic tools that must be employed to reach the set goals.

3.1.1 Morbidity control before PZQ for large-scale use
After the founding of P.R. China, treatment of patients with severe illness and rescuing workforce were the main tasks at the early stages for schistosomiasis control. During the time of the 1950s to the early 1970s, direct fecal examination or the etiologic diagnostic techniques combined with questionnaire were almost compulsory in the identification of individuals for mass chemotherapy because of high infection intensity and toxicity of the available effective drugs for treatment. The strategy of screening a population by ID first followed by stool examination only for the ID positives made large-scale screening feasible and allowed understand the distribution and approximate prevalence of schistosomiasis in a short time during the 1950s. The goal for surveillance was reduced infection intensity and the tools needed should be simple, cheap, sensitive and specific. In parallel with the release of praziquantel for mass drug administration (MDA) in the late 1970s, it was felt that the etiologic or definitive diagnosis resulting from parasitological examination would no longer be needed due to the safety of praziquantel. In addition, the direct parasitological techniques had become relatively insensitive because of lower worm burdens following widespread chemotherapy. The immunodiagnostic approaches were gradually developed and application moved from the laboratory to the field.

3.1.2 Morbidity control during WBLP
3.1.3 Infection control

Once morbidity is under control, the disadvantages of direct stool examination such as insensitivity, heavy work load, decreased compliance of residents providing stool samples become obvious due to the significant decrease of prevalence and infection intensity. More sensitive diagnostic techniques are required. Three Kato-Katz smears from each stool specimens rather than just one started to be used to improve sensitivity of the test (Utzinger et al., 2001, Yu et al., 1998). This approach combined with the immunodiagnostic are currently widely used in the Chinese national control programme as well as for community surveys and field studies, as it is widely acknowledged that single Kato–Katz examinations underestimate the ‘true’ prevalence of *S. japonicum*. The rate of missed infections by the hatching test (7.4-48.8%) turned out to be higher than that of three smear-Kato-Katz (3.1-14.0%) in areas with prevalence rates of ≥5% (Huang et al., 2007b, Xiang et al., 1993). The Kato-Katz ‘missing rate’ increases significantly, and can reach as high as 80%, when the level of prevalence and intensity infection falls to the level characterized by the stage of infection control and transmission-control (Zhu et al., 2005a). When the egg excretion from the host falls below 100 EPG of stool, it becomes increasingly difficult to unequivocally determine whether or not there is an infection (Feldmeier and Poggensee, 1993). Lin et al. (Lin et al., 2008) found that examination of a single thick smear was sufficient when the geometric mean of the faecal EPG was ≥250, while six Kato-Katz thick smears were required when it was lower than 10. The result of three Kato-Katz thick smears would underestimate the true situation when the EPG score was lower than 20.

Although definitive diagnosis of schistosomiasis japonica still relies on the demonstration of viable ova in faeces or histological samples, the immunodiagnostic technology, owing to its rapid, affordable and easily acceptable advantages superior to parasitological techniques, has been integrated into the control program of schistosomiasis in P.R. China as a way of improving the diagnostic record in identifying the target individuals for treatment since the early 1980s. At present, three main types of immunodiagnostic assays are relied on in P. R China. IHA is currently the most widely used immunodiagnostic assay as a screening tool and was extensively used during the World Bank Loan Project (WBLP) period and in nationwide schistosomiasis surveillance sites (Chen et al., 2005, Zhu et al., 2009). Secondly, ELISA was used to estimate the endemic status in the third nationwide sampling survey of schistosomiasis followed by Kato-Katz examination of seropositive individuals (Zhou et al., 2007). Third, rapid diagnostic assays such as DIGFA and DDIA were developed and used in many laboratories (Wen et al., 2005, Zhu et al., 2002).

3.1.4 After transmission under control

When transmission control and transmission interruption have been reached, surveillance becomes more important and particularly so with the move towards elimination. The monitoring cost might increase because control at these levels must
consider different kinds of hosts and require even more sensitive approaches. Now imported cases of infection and a surveillance-response approach must be instituted with enhanced monitoring by antibody detection with highly specific techniques. In the Chinese national schistosomiasis control programme, serology is routinely implemented, usually followed by multiple stool examination (especially the hatching test) of seropositive individuals.

The hatching test is more sensitive than the Kato-Katz technique because the volume of faecal material investigated is several hundred times that used on each Kato-Katz slide, but it delivers only a qualitative assessment that is influenced by factors, such as temperature, pH and the quality of the water. The hatching test is suitable in well-controlled areas and where transmission is supposed to have been interrupted. Occasionally, samples which a negative hatching test may still show eggs by Kato-Katz, particularly if many slides are read. Indeed, numerous studies have dealt with the effect on diagnostic accuracy considering stool consistency, intraspecimen and day-to-day variation in faecal egg output (Booth et al., 2003, Engels et al., 1997a, Engels et al., 1997b, Engels et al., 1996, Enk et al., 2008, Kongs et al., 2001, Utzinger et al., 2001). It thus follows that at least duplicate Kato-Katz thick smears per sample must be analysed not to miss light infections. Another approach to boost diagnostic sensitivity is to employ multiple methods for the same stool sample (Huang et al., 2007a, Zhu et al., 2005a). Some researchers would use three slides each from two samples in order to achieve higher detective rate and more accurate EPG (Xu et al., 2011a), while others (Wang et al., 2009) suggest that the Kato-Katz technique with six smears from two stool specimens can be replaced by three smears from one plus stool the hatching test. Although the sensitivity of the Kato-Katz technique increases with the number of stool samples and slide preparations, it should be remembered that so does the time and manpower required so a system with the primary screen done by serology (IHA) followed by Kato-Katz tests only for the antibody-positive individuals (Balen et al., 2007, Utzinger et al., 2005) was advised from 2005 to 2010. Since 2011, however, the Kato-Katz technique and the hatching test are used in parallel to improve the accuracy (Zhang et al., 2012a).

3.2 Quality control of diagnosis for schistosomiasis control in P. R China

The evaluation of a schistosomiasis control programme requires regular monitoring of the performance of technicians responsible for measuring prevalence rates and intensities of infection, simple quality control measures are the most effective form of monitoring (Braun-Munzinger, 1989). The Kato-Katz technique is frequently used in the field because it is quantitative, inexpensive, and easy to use, but is plagued by decreased sensitivity in areas of low endemicity and particularly in individuals with low worm burdens. It was good idea that the positive and negative accordance rates were evaluated for quality control by standard smears of Kato-Katz. The main external factors influencing the effect were technical ability of operators and quality of microscope in Kato-Katz method. The positive detection rate increased 61.2% by the Kato-Katz method by updating equipment and enhancing training.
(Tokmalaev and Bezborodov, 1982). In 2011-2012, the Kato-Katz technique (three smears from one stool) and the hatching test were evaluated and compared in 81 national surveillance sites in 12 provinces. All of the endemic counties had reached the target with infection control. The results show that the detection efficiency of the hatching test is superior to that of the Kato-Katz technique in the field. However, the levels of the technical personnel for the hatching test are relatively low in most of the surveillance sites (Zhu et al., 2013b). Chen et al (Chen et al., 2011) designed a single-blind method, the stool samples were detected by the hatching method in 2006, 2008 and 2009. The accordance rates of detections in the province-level laboratories for schistosomiasis faeces examination of Zhejiang Province (transmission interrupted in 1995) were 88.9%, 100% and 93.9%, respectively. The laboratories are becoming standardized by means of the repeated training and construction and the stool examination gradually becoming optimized.

The quality of immunology assays is the key of diagnostics for case detection, surveillance and programme monitoring of chemotherapy efficacy and other control interventions. From the 1980s to the 1990s, the assays developed more quickly which was based on the advent of the monoclonal antibody technology for detection of circulating antigens (CAg). Some of these approaches were evaluated through three collaborative studies that took place in 1993, 1995 and 1996. In 1993, eight laboratories participated investigating eight assays using monoclonal or polyclonal probes. The results showed that most of the tests for the detection of different CAg did not perform well producing high numbers of false results ranging from 24 to 46% and showing a low sensitivity ranging from 15 to 73% in chronic and light infection (Yi et al., 1995). In 1995, 14 testing systems, 13 of which aimed for antigen detection and one for antibody detection, from 12 laboratories were evaluated in Wuhan, Hubei Province. The results showed that the specificity of most of the systems tested had been greatly improved compared to the 1993 results with reduced cross-reactions with other helminthic infections and hepatitis. Among the 14 assays, 10 showed high specificity with above 90% but only four assays gave above 60% of sensitivity, the highest of which was 81%, which was in chronic and light infections (Guan and Shi, 1996). In 1996, 12 assays for antigen detection participated in collaborative study again, the results were similar to that in 1995 and the Youden index was more than 0.7 (Feng et al., 1998). Based on the technical levels, the sensitivity and specificity should be improved in order to achieve better results which were no less than 80% and 90%, respectively. The choice of probes was the key of the assay. At the same time, 9 assays for antibody detection participated in parallel. Almost all assays showed high specificity above 90% and sensitivity above 80% in chronic infection (Feng et al., 1998). To supply information for selection of disease detection reagents in the third national schistosomiasis epidemiological sampling survey, another comprehensive evaluation of eight laboratories with nine assays for antibody detection was done in 2004. The results showed most reagents had advanced in quality and were ready to be safely used in field showing sensitivity and specificity above 90% (Xu et al., 2005). However, cross reactions with sera of patients with paragonimiasis were common and
0-30% cross reaction were seen with sera from hepatitis B patients were present in some of the assays (Xu et al., 2005). With the reduced transmission of S. japonicum there is an urgent need for accessible, quality-assured diagnostics for areas of low endemicity. Nine immunodiagnostic tests, developed in P.R. China for the detection of antibodies against S. japonicum, were compared to established their priority for further assessment in field settings (Xu et al., 2011b). All of the tests showed good sensitivities ranging from 92.0% to 98.0% and specificities varying from 70.0% to 97.1%. All tests showed excellent reproducibility with a discordant rate in the range of 0–10.0% for operator-to-operator variation and run-to-run variation. All tests, except one magnetic particle-based ELISA assay, were found to be easy to use and give adequate results, especially the DIGFA. So, there was no clear-cut evidence that the type of antigen-based assays investigated would provide useful correlation to levels of infection intensity, while the antibody-based assays were found to be acceptable for community surveys and screening of people targeted for chemotherapy.

Recent advances in the detection of CAg have solved the problem of insensitivity of this approach. The up-converting phosphate lateral flow (UCP-LF) introduced by (Corstjens et al., 2014, van Dam et al., 2013) makes it possible to detect a single-species worm infection. The UCP-LF CAA assay has been shown to deliver results of previously unimaginable sensitivity and specificity, although further technical improvements will be needed to make it more convenient, simple and applicable for field operation (Corstjens et al., 2014). The UPS-LF was successfully used in the diagnosis of S. japonicum infection in an area of low endemicity in China, using urine samples (van Dam et al., 2015). The assay exhibited a higher sensitivity than that of the Kato-Katz technique and detected a significant number of cases that were egg negative confirming two things: 1) the sensitivity and specificity are sufficient; and 2) there is still considerably more uncured (or reinfected) schistosomiasis in the endemic areas.

4. Current challenges and importance of diagnosis for schistosomiasis control in P. R. China
The prevention and control of the disease need rapid and reliable diagnostic techniques to accurately identify target population for treatment. Current diagnostic technology for schistosomiasis is useful but there are still problems that must be overcome, in particular as we move towards elimination of the disease. Firstly, the stability and quality control of the immunodiagnostic assays must be standardized and strengthened to avoid lower performance in the field than the laboratory (Xu et al., 2007). Before 2005, diagnostic kits for schistosomiasis diagnosis had no national production certificates, which have now started to be issued by the China Food and Drug Administration (CFDA). Although the situation is improving, the quality of immunological diagnosis kits available in the market is uneven (Xin et al., 2006). After a decade of operation, nine immunodiagnostic assays from seven companies have been registered by CFDA (Table 2). Novel approaches, such as PCR and LAMP,
must have standardized production and stable quality control systems testing sensitivity and specificity, including control of storage, transportation and operation. The assays will require simplification of the DNA extraction and field-applicable procedures. Because of the high sensitivity of these two types of assay, attention must be paid to the possibility of false positive by pollution in the laboratory. Importantly, they must be made less expensive.

Secondly, although immunodiagnostics have been extensively, to a certain extent indiscriminately, used in the national schistosomiasis control programme, consideration must be given whether or not available assays are sufficiently good to offer an alternative to the traditional parasitological diagnostic techniques. The currently available antibody-based serological assays cannot distinguish active infection from previous infection or reinfection, which makes it difficult in determining prevalence, identifying infected individuals for selective population chemotherapy and assessing the effectiveness of intervention including follow-up of chemotherapy by this approach. Although the detection of schistosome CAg is regarded as an effective approach to solve the problem but so far this type of assay is unavailable in field, and those that have been tested have an unsatisfactory specificity without sufficient sensitivity, especially in patients with light infections, e.g. chronic cases. According to the results of several collaborative evaluation (see above), there is no information accumulated from the studies carried out to determine whether (1) infection can be assessed qualitatively and quantitatively by serology; (2) clinical morbidity can be correlated with these results; and (3) past and present infection can be differentiated. Work should continue to construct tests based on both antibody or antigen detection, which are duration-dependent and titre-dependent linking with questions, such as discrimination between active and previous infection or reinfection, susceptible versus resistant hosts and identification of pathological consequences. For this reason, the research on defined antigen or antigenic epitopes and corresponding isotype-restricted antibody responses require to be taken seriously.

Thirdly, diagnostic quality is affected by the laboratory conditions, reagents, staff and so on. Feng et al. (Feng et al., 2011) has provided the results of a questionnaire survey for the professionals who are in charge of national surveillance for schistosomiasis, The results show that the average age of laboratory staff was 40.93±9.56 years old; 69% of the personnel were older than 35 years; 86% of them had an education background below the BA degree; staff with primary, middle and high professional titles accounted for 57%, 39% and 2%, respectively. It was pointed out that the equipment of most laboratories needs to be improved and updated. Since 2011, an annual, national technique competition of parasitic disease diagnosis was instituted. Technicians participate from the disease control and prevention perspectives and at the province, prefecture or county levels. The results indicate that the overall level of knowledge of parasitic disease diagnosis has significantly improved owing to the initiated national technique competition. Technicians from provinces with ongoing control activities in schistosomiasis received higher scores than those from provinces
without active control activities. However, some problems still exist, such as the uneven geographical distribution, significant differences in proficiency at identifying different species of parasites, and inadequate coverage (Wang et al., 2015, Zang et al., 2013). Zhu et al. (Zhu et al., 2013a) prepared a specimen panel including four smears with different combinations of eight common helminth eggs and one negative smear, which was examined by 48 personnel from Hubei and Hunan provinces. Out of the participants, 94% correctly recognized schistosome eggs, while fewer than 50% accurately distinguished between the other parasite eggs. Half of the participants misdiagnosed the negative smear. This results clearly shows that the diagnostic capacity should be strengthened and the construction and management of schistosomiasis laboratories standardized.

5. Conclusions

Appropriate diagnostic tools at various stages accelerated the progress of schistosomiasis control in P.R. China. However, there is evidence that the Kato-Katz technique lacks the sensitivity needed at the elimination stage and is therefore unsuitable in areas characterized by low-intensity infections. Although the detection rate increases with examination of several microscopic slides, this reduces the overall cost-effectiveness due to the need for increased work and time. Further disadvantages include the day-to-day fluctuations of faecal egg excretion. Immunodiagnostic techniques, used as an integrated part of the national schistosomiasis control programme, have higher sensitivity and ease of performance but are unable to differentiate between cure and infections. Although molecular methods provide a powerful diagnostic platform they are still expensive and not yet adapted for large-scale field use. Antigen detection remained insufficiently sensitive for a long time but recent advances have been shown one to deliver much improved results. Although further technical improvements will be needed to adopt this approach for field use it has been unequivocally shown to provide superior sensitivity and specificity.

Considering the current global situation with regard to schistosomiasis in the light of the pronounced goal of elimination of the disease, now on the immediate horizon, the reliability of diagnostic tests has become crucial. The research on diagnostic methods should be directed at the production and use of standardized kits with high sensitivity and specificity that are also rapid, simple, non-toxic and inexpensive. It will be important to focus on the target product profiles for the diagnostic tools required for different stages of a schistosomiasis control programme. In addition, to ensure the accuracy and quality of diagnostic work, capacity building and training of professionals should be strengthened, while standard operational procedures (SOP) and quality control for schistosomiasis diagnosis need to be converged in national schistosomiasis laboratory network platforms.
6. References

!!! INVALID CITATION !!!


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