

# Development of a Rapid Diagnostic Kit That Uses an Immunochromatographic Device To Detect Antibodies in Human Sparganosis

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**A diagnostic kit using an immunochromatographic device was developed to replace the time-consuming immunodiagnostic methods for human sparganosis. The kit was found to be faster and easier to use than an enzyme-linked immunosorbent assay (ELISA) and showed higher sensitivity and specificity. It will be useful for the laboratory diagnosis of hospitalized cases of sparganosis.**

Sparganosis is a parasitic zoonosis caused by a larval plerocercoid (sparganum) of tapeworms belonging to the genus *Spirometra*. As the etiologic agents of the disease, *Spirometra erinaceieuropaei* (Rudolphi, 1819) Mueller, 1937 and *Spirometra mansonioides* Mueller, 1935, are important in Asia (1–8) and the Americas (1, 9), respectively.

In the life cycle of the parasite, freshwater copepods are the first intermediate hosts, and frogs, snakes, and chickens are the second intermediate or paratenic hosts. Carnivores, such as dogs and cats, are the definitive hosts. Humans can become infected by consuming raw or undercooked meat from frogs, snakes, or chickens infected with the larval plerocercoid, or by drinking water contaminated with copepods harboring a proceroid, which develops into a plerocercoid (9).

Once a human is infected, the plerocercoid grows and migrates preferentially into subcutaneous tissues and to the abdominal viscera and urogenital organs (1, 5, 6, 9, 10). A potentially painful inflammatory reaction develops in the surrounding tissue, causing the formation of subcutaneous nodules that may appear intermittently (11). The central nervous system and eyes are also affected (5, 11–14).

Although the detection of a plerocercoid is the gold standard method for diagnosing sparganosis, sparganosis is also diagnosed when IgG antibodies specific for plerocercoids are detected in the peripheral blood and cerebrospinal fluid (15–17). Methods for the detection of IgG antibodies have been established, including enzyme-linked immunosorbent assay (ELISA) (15, 18, 19), multidot ELISA (15, 20, 21), immunoblot (22), and two-dimensional immunoblot (23). These methods are time-consuming and require sophisticated equipment. We developed a simple and rapid diagnostic kit (iSpa) using an immunochromatographic device to replace the conventional methods.

To prepare a highly immunogenic cysteine proteinase as a diagnostic antigen (24–26), *S. erinaceieuropaei* plerocercoids were collected from subcutaneous tissues of a Japanese striped snake (*Elaphe quadrivirgata*, family Colubridae) and confirmed to be *S. erinaceieuropaei* by DNA analysis, as reported previously (3). The remaining plerocercoids were treated three times with 10 to 20 volumes of chilled acetone for 20 to 30 min and stored at –20°C.

Since the purification method for the cysteine proteinase was only briefly reported previously (25), we describe the protocol in detail here. The defatted plerocercoids (dry weight, 10 g) were ground and then homogenized in 10 mM Tris-HCl (pH 7.6) containing 0.1 M NaCl, 1 mM EDTA, and 1 mM dithiothreitol (DTT) using a Polytron (Kinematica Co., Lucerne, Switzerland). After centrifugation at 170,000 × g for 1 h at 4°C, the supernatant was treated with 35 to 50% saturated ammonium sulfate to precipitate the cysteine proteinase-rich protein fraction. After centrifugation at 10,000 × g for 1 h, the pellet was resuspended in 5 mM phosphate buffer (pH 7.0) containing 1 mM EDTA and 1 mM DTT and centrifuged at 260,000 × g for 1 h. The supernatant was acidified by adding 50 mM acetate buffer (pH 4.0) containing 5 mM EDTA and 1 mM DTT and incubated at 37°C for 90 min. After centrifugation at 10,000 × g for 1 h, the supernatant was adjusted to pH 6.0 with NaOH and concentrated in 40 to 65% acetone. After centrifugation at 10,000 × g for 1 h, the resultant precipitate was dialyzed against 1 mM NaHCO<sub>3</sub> (pH 7.0) containing 1 mM EDTA and 1 mM DTT and then centrifuged at 260,000 × g for 1 h. A large amount (80 to ~100 mg) of purified cysteine proteinase was obtained from 10 g of defatted plerocercoids.

The human serum samples examined were as follows: parasitologically confirmed sparganosis cases ( $n = 13$ ), other parasitic helminthiases ( $n = 74$ ) that were diagnosed by stool examination and/or serological tests, and healthy volunteers ( $n = 59$ ) who were proven by parasitological and/or serological tests to be free from any parasitic infections. The use of serum samples was approved by the Medical Ethics Committee of the National Institute of Infectious Diseases, Tokyo, Japan (no. 177), and the Human Ethics

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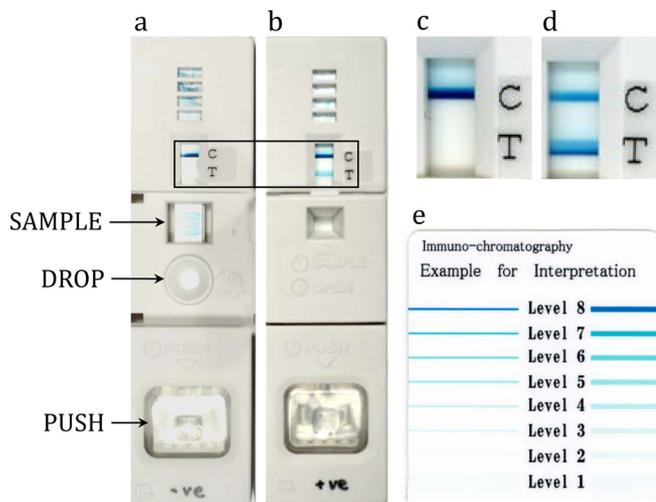


FIG 1 iSpa kit developed in this study. Device with cover open (a) and closed (b). Sample, drop, and push indicate positions for loading of diluted serum sample, conjugate, and substrate solution, respectively. No band appears at test line “T” in the negative case (c), but a blue band becomes visible in the positive case (d). A band at “C” is control line. (e) Reference pattern, levels 1 to 8.

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The iSpa kit using the cysteine proteinase was optimized based on the ELISA results using the cysteine proteinase at Adtech, Inc., Ltd., Oita, Japan. The ELISA was performed according to a previous report (27), except for the use of the cysteine proteinase (0.5  $\mu\text{g}/\text{ml}$ ) and peroxidase-labeled protein G (Zymed, South San Francisco, CA, USA). The kit consisted of an immunochromatographic device, sample buffer, and conjugate. The diagnostic reliability of the kit was evaluated as follows: serum samples were diluted 1:5 in sample buffer, and a 5- $\mu\text{l}$  aliquot of the diluted serum was loaded onto the area inscribed “SAMPLE”. The cover was opened and one drop of peroxidase-labeled anti-human IgG was spotted onto “DROP” and the cover was closed. Finally, a bag inscribed “PUSH” was pressed to release the substrate solution (Fig. 1a and b). A positive result was indicated if a blue band appeared at the test line within 20 min (Fig. 1c and d). The intensity of the blue band was estimated by eye based on supplied reference patterns (Fig. 1e, levels 1 to 8).

The serum samples from 13 confirmed sparganosis cases were all positive, and the sensitivities were 100% in both tests (Tables 1 and 2), although the intensity of the band in the iSpa kit and the optical density (OD) in the ELISA did not always correlate. The specificity of the iSpa kit was calculated as 97.0%, because one case each of neurocysticercosis, ascariasis, fascioliasis, and cerebral paragonimiasis gave a false-positive result. There were no false-positive reactions in the iSpa kit with cutaneous gnathostomiasis and spiruroid larva migrans, which present with clinical symptoms similar to those of sparganosis (Table 1). The paragonimiasis case was a cerebral but not cutaneous form that is much more similar to sparganosis. In contrast, the specificity in ELISA was 86.5% due to the increased false-positive cases. These false-positive reactions were likely to be observed when the antibody levels were higher ( $\geq 0.8$  OD in ELISA). The positive predictive value (76.5%) for the iSpa kit was higher than the value (41.9%) in

TABLE 1 Serum samples examined and diagnostic results by iSpa kit and ELISA

Serum samples from patients with <sup>a</sup>	No. of positive cases/no. of cases examined for:	
	iSpa kit	ELISA
Sparganosis	13/13	13/13
Gnathostomiasis	0/13	1/13
Cysticercosis	1/10	5/10
Ascariasis	1/5	1/5
Capillariasis <sup>a</sup>	0/5	0/5
Anisakiasis	0/2	0/2
Spiruroid larva migrans	0/1	0/1
Strongyloidiasis	0/9	2/9
Trichinellosis	0/2	1/2
Dirofilariasis	0/3	0/3
Onchocercosis	0/1	1/1
Loasis	0/2	0/2
Fascioliasis	1/5	4/5
Paragonimiasis	1/16	3/16
Healthy individuals	0/59	0/59

<sup>a</sup> Capillariasis due to *Capillaria philippinensis*, spiruroid larva migrans due to *Crassicauda giliakiana* (36), trichinellosis due to *Trichinella papuae* (37) and *Trichinella spiralis* (38), dirofilariasis due to *Dirofilaria immitis*, onchocercosis due to *Mansonella perstans*, fascioliasis due to *Fasciola gigantica*, paragonimiasis due to *Paragonimus heterotremus*, *Paragonimus miyazaki*, and *Paragonimus westermani*.

ELISA at the prevalence of 8.9% (13/146). This indicates that the kit is more applicable for groups with a high prevalence, e.g., hospitalized cases.

Although sparganosis cases in which the antibody levels were monitored over a long period are limited, we tried to monitor antibody levels using two diagnostic tools. Figure 2 shows the change in antibody levels monitored over 2 years after surgical treatment in a cerebral case (28). The antibody level was temporarily elevated after a craniotomy, but the levels decreased 5 months later and then gradually declined. It has been reported that antibody levels decline after 3 to 10 months in sparganosis cases (17, 29–31), and the kinetics of the antibody response after treatment may depend on individual patients and/or tissues affected by plerocercoids. The iSpa kit may be useful for monitoring the time course of recovery.

Sparganosis is a neglected parasitic disease, and there are few public health strategies aimed at the prevention and control of the

TABLE 2 Comparison of parasitological and immunological diagnostic methods for sparganosis

Test type and results	No. with plerocercoid presence		Total no.
	+	–	
iSpa kit			
+	13	4	17
–	0	129	129
Total	13	133	146
ELISA			
+	13	18	31
–	0	115	115
Total	13	133	146

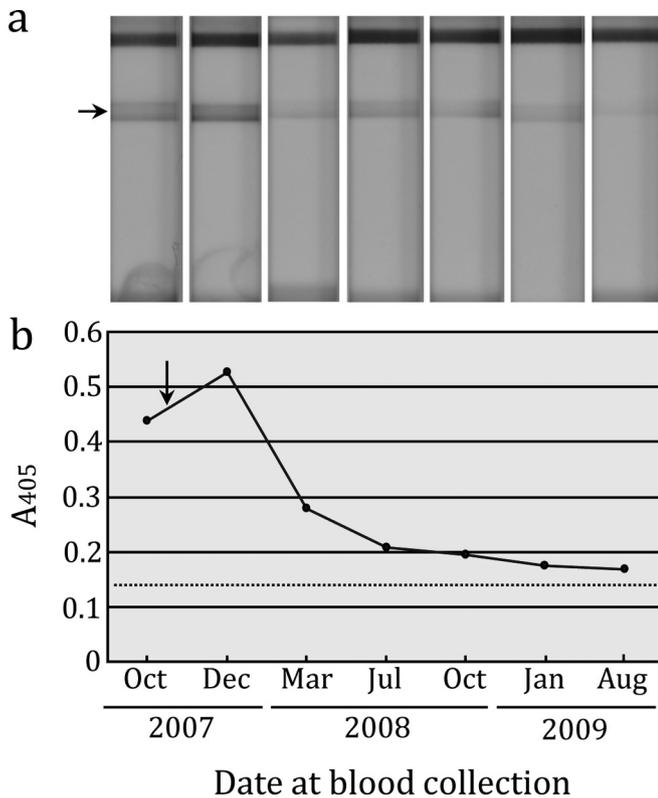


FIG 2 A follow-up case of cerebral sparganosis using iSpa kit and ELISA. Shown are the results of the changes in antibody levels using the iSpa kit (a) and ELISA (b). (a) Arrow, positive bands appeared at dates when blood samples were taken. (b) Dotted line, cutoff value (0.133); arrow, craniotomy performed on 31 October 2007.

infection; however, immunodiagnostic methods have been well established (15, 18–23). Although the definitive diagnosis of sparganosis is the detection of plerocercoid, there have been reported cases of sparganosis in which plerocercoids were not detected and the diagnosis was based on a decrease in antibody titers and eosinophil counts, as well as an improvement in clinical symptoms after chemotherapeutic intervention (32, 33). Therefore, a sparganosis diagnosis should be considered if an antibody against the plerocercoid is detected even in the absence of plerocercoids.

In this study, a native cysteine proteinase was used as the diagnostic antigen. A recombinant cysteine proteinase is also available (34, 35), and both antigens can be easily produced in large amounts and may show high sensitivity and specificity compared to those of native antigens (35).

In conclusion, the iSpa kit has several advantages compared with conventional methods. It is a simple and rapid (~20-min) kit that does not need any technical experience or sophisticated equipment. The increased sensitivity and specificity of the kit provide a more reliable diagnostic result. It is expected that the iSpa kit will be a useful tool for laboratory diagnosis and for suspected cases for which biopsy and excision procedures are not feasible.

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