International Commission on Trichinellosis: Recommendations on the Use of Serological Tests for the Detection of *Trichinella* Infection in Animals and Man

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Summary

The use of serological tests to detect *Trichinella* infection in domestic and wild animals and in humans has not been standardised yet. This review provides an uniform set of recommendations for the development and use of serological tests to detect circulating antibodies in serum samples. The recommendations are based on the best scientific published information and on the unpublished data from laboratories with a great expertise in this field and represent the official position of the International Commission on Trichinellosis regarding acceptable methods and the evaluation of the sensitivity and specificity. These recommendations are subject to change as new scientific information becomes available.

Key words: *Trichinella*, trichinellosis, serodiagnosis, animals, humans, antigens, specificity, ELISA, sensibility
Introduction

According to the International Commission on Trichinellosis (ICT) (Gamble et al., 2000; www.med.unipi.it/ict/welcome.htm) indirect (serological) methods are not recommended for the testing of individual carcasses of food animals at slaughter. This recommendation is consistent with practical legislation of many governmental bodies, under which meat inspection programs for *Trichinella* in pork, horse and game meats are performed using direct methods including trichinoscopy and artificial digestion (see Gamble et al., 2000; Nöckler et al., 2000; European Commission, 2001). Legislation for inspection of meat and meat products for the presence of *Trichinella* includes direct tests only, which if performed properly, have the sensitivity and specificity sufficient to detect all carcasses with larval burdens ≥ 1 per gram of meat, the minimum level of infection considered to be of public health concern. No serological methods should be included in legislation for meat inspection because these methods do not meet the required standards for protection of public health.

Although serological methods for detection of *Trichinella* infection are not considered to be suitable for the purpose of meat inspection, there are important uses for these tests including: 1) surveillance of infection and epidemiological investigations in animal populations, particularly where the prevalence is high; and, 2) presumptive or retrospective diagnosis and surveillance of human infection. Due to the various methods that have been employed for serological detection of *Trichinella* infection, the ICT has compiled a series of recommendations for the use and interpretation of these tests.
SEROLOGICAL METHODS

TYPES OF ASSAYS

Many types of serological assays have been, and continue to be, used for the detection of *Trichinella* infections in animals and man. For animals, serological assays include, but are not limited to: 1) indirect immunofluorescence assay (IFA) employing cryostat sections of infected rodent muscle, frozen sections of free muscle larvae or formalin fixed whole larvae; 2) immuno-electrotransfer blot assay (IETB), or western blot, using muscle larvae excretory-secretory products as antigen; 3) enzyme immunohistochemical technique (EIH) employing cryostat sections of infected rodent muscles (e.g., diaphragm) or frozen sections of free muscle larvae; and 4) enzyme-linked immunosorbent assay (ELISA) employing the excretory-secretory (ES) products of the muscle larvae (Gamble *et al.*, 1983; 1988) or synthetic tyvelose as antigen (Gamble *et al.*, 1997; Kapel and Gamble, 2000; Pozio *et al.*, 2002a). For detection of human infection, indirect hemagglutination (IHA), bentonite flocculation, IFA, latex agglutination and the ELISA are the more commonly used tests, the last being the most sensitive (Murrell and Bruschi, 1994; Dupouy-Camet *et al.*, 2002). Competitive inhibition assay (CIA) and IETB (Dupouy-Camet *et al.*, 1988; Ivanoska *et al.*, 1989; Homan *et al.*, 1992; Pozio *et al.*, 1993, 1997, 2002a; Owen *et al.*, 2001; Pinelli *et al.*, 2001) have also been used for the diagnosis of trichinellosis in humans. These tests are considered confirmatory tests, rather than primary screening tests.

The ELISA is the most commonly used method for the detection of *Trichinella* infection in both animals and man because it is economical, reliable, readily standardized and provides an acceptable balance of sensitivity and specificity. It is the only serological method recommended by the Office International des Epizooties (OIE, 2000) for testing domestic
pigs. For these reasons, the ELISA will be the primary focus of these recommendations. However, other types of tests can have practical applications; therefore, the principles for use of the ELISA (requirements for performance, suitability for particular species, etc.) should be points for consideration in selecting any serological test for detection of *Trichinella* infection. For the ELISA, and for any serological test, independent validation should be performed using sera from infected and *Trichinella*-free animals or humans representative of the local population where the test is being used. The cut-off value of the ELISA, for example, can change based on host genotype and exposure to antigens in the local environment and in food. These are important considerations when testing either animals or humans (Navarrete *et al.*, 1989). Control positive and negative sera are required on every ELISA plate to assure the test is performing properly (OIE, 2002).

**ANTIGENS**

In the 1970s somatic antigens prepared from whole body extracts of muscle larvae were used in the ELISA for detection of *Trichinella* antibodies in pigs (Ruitenberg *et al.*, 1974; Ruitenberg and van Knapen, 1977). This crude larval extract, produced from first stage muscle larvae collected by artificial digestion, should not be used for serological tests of either animals or humans due to a high probability of cross-reactions with other pathogens. For example, cross-reactions occur with serum samples from humans infected with *Loa loa*, *Toxocara* sp., and *Anisakis* (J. Dupouy-Camet, personal communication; E. Pozio, unpublished data).

During the 1980s, the specificity of the ELISA was improved by utilizing excretory-secretory (ES) antigens obtained from the *in vitro* incubation of *Trichinella* muscle larvae (Gamble et
al., 1983). These antigens, originating from larval secretions, consist of a group of structurally related glycoproteins. Information regarding *Trichinella* antigens has been summarized by Appleton *et al.* (1991) and Ortega-Pierres *et al.* (1996). The predominant antigen epitope recognized by animals and humans infected with *Trichinella spiralis*, or any of the other species of *Trichinella* currently known, is the so-called TSL-1 group. TSL-1 antigens are found in the stichocyte cells and on the surface of the parasite’s cuticle; they are actively secreted by first-stage larvae in the muscle. These antigens are produced for diagnostic use by *in vitro* cultivation of isolated muscle larvae or by biochemical methods of recovery from this same parasite stage. Methods for the preparation of these antigens have been published (Gamble *et al.*, 1983; Seawright *et al.*, 1983; Grencis *et al.*, 1986; OIE, 2000) and the reader is referred to these articles for a detailed description of the methods.

As noted, the TSL-1 antigen epitopes recognized by antibodies in *Trichinella*-infected animals and humans are common to all encapsulated and non-encapsulated species (Appleton *et al.*, 1991). Consequently, *Trichinella* antigen can be routinely prepared from *T. spiralis* muscle larvae, because this species is readily maintained in laboratory mice, and used for ELISA to detect infection with any *Trichinella* species.

TSL-1 antigens share a common carbohydrate epitope (tyvelose), which has been synthesized (Reason *et al.*, 1994; Wisnewski *et al.*, 1993). Synthetic tyvelose is available commercially (Safe-Path Laboratories, LLC, Carlsbad, CA), and has been evaluated in several research studies, where its performance compared favorably with ES antigens for testing humans, pigs and other animals (Gamble *et al.*, 1997; Bruschi *et al.*, 2001; Owen *et al.*, 2001; Pozio *et al.*, 2002a). The synthetic carbohydrate antigen offers the advantages of stability and standardization. It has been shown to provide greater specificity, in many species, but may
sacrifice sensitivity. Recent reports of possible cross-reactions obtained using the tyvelose antigen and evidence of tyvelose-containing glycans in other nematode eggs will necessitate further studies to better define its specificity (Dea-Ayuela et al., 2001; Pozio et al., 2002a).

REAGENTS

Conjugates - Better specificity is obtained in the ELISA and IETB using a species-specific anti-IgG reagent as compared with a Protein A conjugate. It is strongly recommended that a species-specific anti-IgG reagent be used even if it is necessary to have it custom made.

SAMPLE COLLECTION

Serum is the preferred sample for conducting serological tests for *Trichinella*. Other samples that have been used include plasma, whole blood, and tissue fluids (Gamble and Patrascu, 1996). After collection, blood samples should be clotted and the sera frozen at −20°C as soon as possible; samples frozen at −20°C may be used for several months. It is important to avoid repeated freezing and thawing of samples, since serum titers will decline as a result; therefore, samples that will be used frequently should be made into aliquots. For periods of storage greater than 3 months, serum samples should be frozen at −80°C or lyophilized. If freezing is not possible, 1% merthiolate, or another suitable preservative, should be added to each serum sample at a dilution of 1:10,000.

Another alternative method for sample collection and storage is blood spots on filter paper (Owen et al., 2001). This method is useful when there are no facilities to collect blood using a syringe; blood spots may be stored at room temperature.
For tests performed using animal carcasses, where blood or serum is not available, tissue fluids or meat juice samples are an alternative. Several studies have reported on the use of tissue fluids for detection of *Trichinella* infection (Gamble and Patrascu, 1996; Kapel et al., 1998). Generally samples of tissue fluids are used at a lower dilution (higher concentration) in serological assays. If meat samples are used for the extraction of tissue fluids it is recommended to wash the tissue, cut it into small pieces, freeze and thaw it and use these new extracts instead of muscle fluids found in an original sampling, which is often mixed with blood and/or other surface contaminants.

**VALIDATION AND QUALITY CONTROL**

An acceptable serological assay is one that is properly standardized and validated for its intended purpose. All components of the test that are critical for maintaining suitable performance (critical control points) should be identified and appropriately controlled, and the test should be conducted within a laboratory quality system (Gajadhar and Forbes, 2002; OIE, 2002). In particular, each batch or lot of antigen should be checked by checkerboard titration against a standardized positive control serum.

Commercially available serological tests such as the ELISA often must meet specific standards of sensitivity and specificity established by an individual country’s licensing authority. Users of commercial tests should verify that the test has been adequately evaluated using international reference standards and has received the approval of any relevant regulatory authorities. Commercial test kits that are not regulated often yield results that include high numbers of false positive (quite frequently) and false negative (less frequently) results. The user of any test should always conduct an independent evaluation of test
performance using panels of defined positive and negative sera that are representative of the population to be tested. The performance of the test relative to specificity and sensitivity should meet the purpose of the test.

When intended for use in epidemiological studies, the sensitivity and specificity of the ELISA, and other serological tests, must be considered when interpreting results. Estimates of sensitivity and specificity can be obtained by testing adequate numbers of known positive and known negative animals or humans. The negative control group should be representative of the local population and positives should represent various stages and levels of infection. For humans, blood donors often represent a good source of reference serum samples. The Office International des Epizooties (OIE, 2000) recommends a minimum of 300 known positive and 1000 known negative samples to establish sensitivity and specificity.

For a comprehensive discussion of requirements for the validation of a serological test, the reader is referred to the OIE (2000; 2002) (www.oie.int/eng/normes/mmanual/A_00013.htm).

RECOMMENDED USES

SURVEILLANCE IN FOOD ANIMALS

1. Swine

   Suitability of test

   The ELISA test, using ES or tyvelose antigens, is useful for surveillance systems to estimate the prevalence of infection in a domestic pig population. When possible, serologically positive animals should be examined by artificial digestion to confirm infection, to estimate the worm burdens, and to identify the species of *Trichinella*. 
Artificial digestion using an appropriate amount of tissue (i.e., 100 grams or more) should be performed according to ICT Recommendations (Gamble et al., 2000; www.med.unipi.it/ict/welcome.htm).

Serological methods, including the ELISA, should not be used for the detection of *Trichinella* infection in individual carcasses at the slaughterhouse, for the purpose of protecting human health.

Sensitivity and specificity

In pigs, the sensitivity of the ELISA, using an ES antigen, has been reported to range from 93.1 – 99.2%, provided sufficient time has elapsed for infected animals to develop an antibody response (see Interpretation of Results, below). The specificity of the ES ELISA in pigs has been reported to range from 90.6 – 99.4% (Murrell et al., 1986; Oliver et al., 1989; van der Leek et al., 1992). Infections of as few as 1 larva per 100 g of tissue can be detected by ELISA (Gamble et al., 1983; Kapel and Gamble, 2000).

The use of a somatic extract of *Trichinella* (e.g., Melcher’s antigen) in the ELISA results in false positive reactions with sera from pigs infected with other parasites and/or microbes. Several studies have demonstrated that both ES and tyvelose antigens reduce cross-reactions that occur when using a crude *Trichinella* antigen in the ELISA (Gamble et al., 1983; 1988; 1997). The quality of ES antigen depends on adhering to proper methods for the cultivation of *Trichinella* muscle larvae and proper purification of the antigen (Gamble et al., 1988).
Methodologies

A general method for conducting an ELISA test in pigs is described in the OIE Manual of Standards for Diagnostic Tests and Vaccines (Chapter 2.2.9) (Gamble, 2000) (www.oie.int/eng/normes/mmanual/A_00013.htm).

By special arrangements, standard antigens, reference sera and scientific consultation can be obtained from ICT members’ laboratories (www.med.unipi.it/ict/welcome.htm) and the OIE Reference Laboratories (www.oie.int/eng/oie/organisation/en_listeLR.htm).

Interpretation of results

The level of infection of pigs with *Trichinella* larvae (worm burden) is directly correlated with the time required for antibodies to appear in the blood. For light infections (< 1 larva per gram of tissue), antibodies might not be detected by ELISA for 4-7 weeks or more following exposure (Gamble *et al.*, 1983; Gamble, 1996, 1998), while antibodies might be detected from 2.5-3 weeks in pigs with higher numbers of muscle larvae (Smith and Snowdon, 1989; Kapel and Gamble, 2000). Thus, there is a risk of obtaining false-negative results during the early stages of infection, and for this reason, the ELISA and other tests measuring a serological response cannot be used for individual carcass testing.

On the other hand, there is no correlation between the ultimate worm burden (larvae per gram of tissue) and the result of the ELISA test once an animal has become
serologically positive. Therefore, artificial digestion of tissue is an important adjunct to ELISA to determine the public health risk associated with infected animals.

*Trichinella* antibodies may persist in pigs for a long time (Nöckler et al., 1995). It can be assumed that in slaughter pigs, which have a live weight of 90 to 100 kg at an age of 25 to 30 weeks, it is unlikely that a false-negative result will result from declining antibody titer (Nöckler et al., 2000).

2. Horses

Suitability of the test

Studies in horses have shown that serological responses to *Trichinella* infection in this species are less consistent than responses observed in pigs, rodents and humans (Smith and Snowdon, 1987; van Knapen et al., 1987; Polidori et al., 1989; Soule et al., 1989; Gamble et al., 1996; Voigt et al., 1997; Yepez-Mulia et al., 1999; Sofronic-Milosavjevic et al., 2001; Pozio et al., 2002a). Studies in horses experimentally infected with 5,000 *Trichinella spiralis* larvae reported a decline in antibody titer at week 15 post-infection, at which time infective larvae were still present in the muscle tissue (Voigt et al., 1997).

Low specificity with ES antigen and tyvelose antigen and low sensitivity due to a decreasing antibody titer, even in the presence of high numbers of larvae in the musculature, have been reported using ELISA and IETB (Soule et al., 1989; Pozio et al., 1997, 2002a).
Considering the present state of knowledge, the ICT cannot recommend serological methods for use in horses either for detection of single infections or for reliable surveillance. However, further studies are needed, especially in endemic regions, to determine if serological methods might have some value.

Special considerations
A recent report (Pozio et al., 2002a) suggested the possibility that *Trichinella* has antigens in common with some plant parasitic nematodes, including tyvelose, which is believed to be highly specific (Spiegel and McClure, 1995; Lopez de Mendoza et al., 1999). If this report is substantiated in further studies, it will pose further problems for serological detection of *Trichinella* infection in the horse and more generally in herbivores.

3. Game and other animals intended for human consumption

Suitability of the test
Relatively few studies have used serological methods for estimating *Trichinella* infection in game animals intended for human consumption. There have been a few field studies estimating *Trichinella* infection in wild boars using an ES antigen in an ELISA. From the Netherlands, 31 of 458 samples (6.8%) were positive (van der Giessen et al., 2001) and from Germany, 234 of 16,888 samples were positive, corresponding with a seropositivity of 1.4% (Nöckler et al., 1999). This seroprevalence is much higher (up to 100 times) when compared with the prevalence of *Trichinella* in wild boars as determined by artificial digestion (between 0.001 and 0.01 % in Germany). These findings might be interpreted as resulting from the higher
sensitivity of the ELISA or a lower specificity of the ELISA due to cross-reactions. Based on these aforementioned studies, insufficient information is available on the performance of the ELISA in game animals to recommend this method for individual carcass inspection or for epidemiological studies for the purpose of estimating true prevalence. Further studies are required to determine the influence of cross-reactions and quality of blood samples on the specificity of the ELISA and also to determine whether very low level infections may be responsible for some of the positive serological results obtained.

Game animals intended for human consumption should be examined by artificial digestion, according to approved methods (Gamble et al., 2000; www.med.unipi.it/ict/welcome.htm).

SURVEILLANCE IN OTHER ANIMAL SPECIES

Suitability of the test
Due to the high number of parasitic, bacterial, fungal and viral infections, which can be present in wildlife, the risk of cross-reactions with non-specific antibodies is very high.

Therefore, the use of serological methods for determining prevalence of *Trichinella* infection in wildlife must be preceded by a thorough evaluation of test sensitivity and specificity in the species and population being tested. The ICT discourages the use of serological methods for the detection and surveillance of *Trichinella* infection in wildlife, without such an evaluation. Any evaluation should include digestion of a minimum of 100 grams of tissue.
It is likely that some reports of serologically positive animals are the result of false positive reactions. This caution on the use of serological methods for testing wild animals is suggested by studies that reported a high serological prevalence for *Trichinella* in animals without larvae in muscles from regions where *Trichinella* infection was never observed (Vercammen *et al.*, 2002) or the prevalence rate detected by artificial digestion was 100 times lower than that recorded using serological methods (Nöckler and Voigt, 1997a; Wacker *et al.*, 1999). However, the much greater sensitivity of ELISA might also be a factor when testing wildlife populations where worm burdens may be very low (only a few larvae per kilogram of tissue). Additional studies are required to assess the occurrence of false positive reactions and the detection of very low level infections by serological methods in wildlife populations. To assure prevention of human disease, any meat-eating game animals intended for human consumption should be examined by tissue digestion.

Sensitivity and specificity

Sensitivity and specificity should be determined by testing panels of sera from a sufficient number of known positive and known negative animals representing the species and population, prior to using a test. Since this is often not possible with wildlife species, the limitations should be clearly understood and explained when reporting or interpreting results.

Methodologies
1. Wild boar - In one study, an antibody response was detectable by ELISA 3 to 4 weeks after experimental infection of wild boars with different encapsulated *Trichinella* species (Kapel, 2001), however, seroconversion was delayed in infections with non-encapsulated species. The antibody level remained stable in wild boars infected with *T. spiralis*, *T. britovi* and *T. nelsoni*, but the rapid decrease of antibodies directed against *T. nativa* and *Trichinella* T6 was apparently associated with the rapid disappearance of larvae.

A few epidemiological studies have used ES antigens for the evaluation of prevalence in wild boar populations (van der Giessen *et al.*, 2001), but these prevalence results have in general been much higher as compared to results obtained by artificial digestion. In contrast, the experimental studies of Kapel (2001) have shown a good correlation between larval burden and antibody response. Such differences are probably related to cross reactions resulting from infection with other tissue-migrating nematodes (*Toxocara, Ascaris, Spirura*), but no studies have been conducted to evaluate this phenomenon in wild boar.

*Bear* - Several studies have reported the use of ELISA to detect infection in bears (*Ursus arctos, Ursus americanus*) (Zarnke *et al.*, 1997; Nutter *et al.*, 1998); however, confirmatory digestion testing did not correlate well with serological results. Better specificity in the ELISA can be obtained by using a customized anti-bear IgG reagent as compared with the Protein A conjugate in the ELISA test (Gamble H.R., unpublished data).
Fox - Experimental studies in silver foxes (Vulpes vulpes) showed that use of an ES antigen in an ELISA is a suitable method for serological detection of Trichinella infection in this species (Kapel, 2000). Specific antibodies were detectable in all foxes infected with 500 or 2000 larvae between 2 and 6 weeks post infection, and these antibodies persisted throughout the experiment (30 and 76 weeks post-infection) with mean larval recovery rate at post mortem ranging from 4.3 to 11.3 larvae per gram of tissue (Nöckler and Voigt, 1997b). Anti-dog-IgG peroxidase conjugate is suitable as an alternative to the species-specific anti-fox antibody system since there is a close phylogenetic relationship between foxes and dogs. Results from field studies demonstrated a 100 times higher Trichinella seroprevalence in the red fox population (7.7%) compared to a prevalence of 0.07% in foxes examined by artificial digestion (Wacker et al., 1999). As in wild boars the huge difference between seroprevalence and actual detection of parasites may be due, in part, to the higher sensitivity of ELISA (0.01 larvae per g) and the limited ability to detect muscle larvae in small samples of muscle tissue. It should be considered that the larval recovery rate in the majority of foxes might be very low, i.e. about or less than 0.1 larvae per g of musculature, as published by Enemark et al. (2000) and van der Giessen et al. (2001).

Special considerations
The variability of collection methods for game meat samples often creates problems in conducting serological tests. Samples are frequently contaminated by bacteria or fungi, or they may be hemolyzed; these problems can cause false positive results. Filtration of contaminated samples with a 0.22 µm filter is useful to reduce the background, but is not always entirely effective.
Interpretation of results

False negative results have been observed in wild animals (e.g., foxes and wild boars) with long-term low-level infections with sylvatic species, (e.g., *T. britovi*) (Pozio E., unpublished data).

**USE IN DIAGNOSIS OF HUMAN TRICHINELLOSIS**

According to Ljungström (1983) there are three objectives in the immunodiagnosis of human trichinellosis: (a) recognizing the acute infection to allow early anthelminthic treatment; (b) making a retrospective diagnosis; and (c) adding information to the epidemiology of the infection.

Suitability of test

Many serological tests are available for human diagnosis (Despommier, 1986; Murrell and Bruschi, 1994; Kociecka *et al.*, 2001; Dupouy-Camet *et al.*, 2002). These tests include, for example, ELISA, IFA, CIA, IETB and counterimmunoelectrophoresis (CIE). Diagnostic laboratories might use more than one test for screening; for example, screening by ELISA to detect the response against a soluble antigen and by IFA to detect antibodies that react with antigens on the larva’s cuticle. When a single preliminary serological test is positive, another test, such as an IETB or CIA might be performed for confirmation. As a general rule, however, the ELISA with ES or tyvelose antigen can be used as the primary diagnostic of choice to detect infections in humans.
Sensitivity and specificity

Absolute sensitivity (100%) has been obtained in humans infected with *T. spiralis* by ELISA (measuring IgG) using both a crude larval extract or ES antigens (van Knapen *et al.*, 1982, Bruschi *et al.*, 2001). This high rate of sensitivity, observed 50 days after infection, declined to about 80% after two years. However, parasite-specific IgM was detected for up to 11 years after infection. Serological tests measuring other classes of antibodies (IgA, IgE) resulted on lower sensitivity rates (Murrell and Bruschi, 1994). The study of the humoral response against stage-specific antigens has not resulted in an improvement in serodiagnosis; however, the search for newborn larvae specific IgA has shown promise for use in detection of the early phase of the infection (more than 80% of positivity after three weeks of infection) (Mendez-Loredo *et al.*, 2001).

The sensitivity of ELISA was reported to be higher with ES antigen than with tyvelose for detecting circulating antibodies early in the infection (E. Pozio, unpublished data). However, in another report, asymptomatic infections are detected using the tyvelose antigen (Owen *et al.*, 2001). Additional comparative studies are needed with these two antigens. High sensitivity (up to 100%) was also obtained with other tests, including IFA (Pozio *et al.*, 1988) and CIA (Ivanoska *et al.*, 1989).

Specificity depends primarily on the type of antigen used and the cut-off value established. Using the ELISA (Engvall and Ljungström, 1975), ES antigens are preferable to crude extracts of *T. spiralis* muscle larvae, since the former resulted in a higher specificity. This is particularly important in regions where other human
helminth infections (e.g., *Ascaris, Trichuris*, filariae) are common and cross-reactions with these parasites could give false positive results (Au *et al.*, 1983; Mahannop *et al.*, 1995). In industrialized countries, the risk of cross-reactions using ES antigen is low, but cross-reactions do occur with human larval migrans of unknown species. In sera from Greenlanders, false-positive reactions were suspected to be due to infection with *Anisakis* or *Pseudoterranova* from ingestion of marine fish (C. Kapel, unpublished data).

The use of the synthetic tyvelose antigen in the ELISA has resulted in even greater specificity (Bruschi *et al.*, 2001; Owen *et al.*, 2001), although some exceptions have been noted (Dea-Ayuela *et al.*, 2001). Further study on this synthetic antigen is required.

Western blots have shown promise for discriminating trichinellosis patients from patients with other helminth infection, although possible cross-reactions may occur with cases of anisakiasis (Yera *et al.*, 2003) and schistosomiasis (Dupouy-Camet J., unpublished data).

Methodologies

Indirect hemagglutination (IHA), bentonite flocculation (BFT), IFA, latex agglutination (LA), and ELISA are the more commonly used tests, the last being the most sensitive (Murrell and Bruschi, 1994; Kociecka, 2000). CIA (Ivanoska *et al.*, 1989) and IETB (Dupouy-Camet *et al.*, 1988; Pozio *et al.*, 1993; Owen *et al.*, 2001)
have also been used. The ELISA for human infection is performed essentially as
described for pigs, with the substitution of an anti-human IgG reagent.

IFA can be carried out either using frozen sections of infected tissue (Ljungström,
1974) or formalin fixed whole larvae (Brzosko et al., 1965; Pozio et al., 1988), as antigens, the former being more sensitive. With this test, all relevant immunoglobulins can be detected. Cross-reactions with *Trichinella* antigens were observed in persons with autoimmune diseases (Robert et al., 1996). Persons reading the fluorescent sections should be aware that only sections with a uniform fluorescence should be considered as positive, whereas those, which show a non-uniform fluorescence along the cuticle, should be considered as false-positive. Counter staining with Evans Blue reduces false positive results and facilitates reading of slides (Boireau et al., 1997).

IETB can be used as a primary or confirmatory test and when ES antigens are used it is quite specific and useful for follow-up studies (Andrews et al., 1995). The presence of antibodies specific for the TSL-1 antigen family (40-70 kDa in the reduced form) should be considered diagnostic.

Interpretation of results
For humans, as for animals, it is imperative to evaluate the cut-off value on the basis of a panel of serum samples (at least 100-200 sera), which are representative of the human population for which the test will be used. The human genotype, food habits and environmental characteristics can influence the background of a serological test. This preliminary evaluation should be done for both commercial kits and laboratory-
developed tests. It is also important that the cut-off value be confirmed, every time the antigen, reagents or materials (e.g., ELISA plate) are modified or substituted.

Antibody levels do not correlate with the severity or the clinical course of trichinellosis in humans (Murrell and Bruschi, 1994). Seroconversion usually occurs between the second and fifth week of infection and the time required for seroconversion is inversely correlated with the infective dose. Serum may remain positive up to 1 year or more (19 years has been reported) after the end of the acute phase of infection (Pozio et al., 1993). In *T. britovi* human infections, seroconversion has been documented up to two months post infection. Serological testing performed in a large outbreak of human trichinellosis due to *T. nativa* revealed a seropositive rate of 45% and 87% at 3-4 and 10-11 weeks post-infection among confirmed cases. However, seroconversion from acute to convalescent samples was 55% (Schellenberg et al., 2003).

An evaluation of the antibody kinetics in infected persons should be conducted every three months to follow the success of chemotherapy. In persons infected with *T. britovi*, circulating antibodies disappear (in about one half of patients) within six months and within three years all persons are seronegative (Pozio et al., 1993). In *T. spiralis* infected persons, who received a delayed or ineffective therapy, specific antibodies can be detected for years (Pozio et al., 2001), whereas in those who received effective treatment in the first two weeks after infection specific antibodies can disappear in a shorter period of time.
The IFA has proved to be the most reliable method for discriminating between patients with biopsy-confirmed infection and those patients suspected of exposure, but biopsy negative (Murrell and Bruschi, 1994). Due to the fact that IFA is based on cuticle surface antigens of muscle larvae, cross-reactions may occur with *Onchocerca* spp. and *Schistosoma mansoni* (Robert *et al.*, 1996).

**SPECIES IDENTIFICATION**

According to current taxonomy, the genus *Trichinella* is composed of eight distinct species, all of which can infect humans (Pozio E., 2001; Pozio *et al.*, 2002b). Of these eight species, five have an encapsulated larva in host muscles and infect only mammals (*T. spiralis*, *T. nativa*, *T. britovi*, *T. murrelli*, and *T. nelsoni*) and three show non-encapsulated larvae in host muscles and infect both mammals and birds (*T. pseudospiralis*) or mammals and reptiles (*T. papuae* and *T. zimbabwensis*) (Pozio *et al.*, 2004). Furthermore, other genotypes strictly related to the above reported species have been described. Specifically: *Trichinella* T6 is related to *T. nativa*, *Trichinella* T8 and *Trichinella* T9 are related to *T. britovi* (Pozio, 2001). All of these species and genotypes can be easily identified by a multiplex-PCR and PCR-RFLP (Wu *et al.*, 1999; Zarlenga *et al.*, 1999; Pozio and La Rosa, 2003).

Serological methods cannot be used to differentiate species. Although minor differences in the immune response against different *Trichinella* species have been described (Pozio *et al.*, 1993; Bruschi *et al.*, 1999; Kapel and Gamble, 2000) these differences were generally related to the kinetics of the antibody response. The antibody response is dependent on the rate of production of newborn larvae, which is generally higher in *T. spiralis* as compared with other species.
Differences have been observed in ELISA optical density values or IETB patterns obtained using serum samples of animals (Kapel and Gamble, 2000; Kapel, 2001) and humans (Pozio et al., 1993) infected with a Trichinella species which is the same or different from that which was used to prepare the antigen. However, these differences are of limited value for diagnostic purposes.

CONCLUSIONS
This guideline is based on the best scientific published information and on the unpublished data from laboratories with a great expertise in this field and represent the official position of the International Commission on Trichinellosis regarding acceptable methods and the evaluation of the sensitivity and specificity. These recommendations are subject to change as new scientific information becomes available.

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