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Evaluation of a commercial immunochromatographic assay for the serologic diagnosis of tularemia

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ABSTRACT

Tularemia is an infection caused by *Francisella tularensis* with a worldwide distribution in the northern hemisphere and diverse clinical manifestations. Serology plays an important role in the diagnosis of tularemia. A commercially available immunochromatographic assay (ICA) for the serologic diagnosis of tularemia (VIRapid Tularemia, Vircell, Granada, Spain) was evaluated, and the performance was compared with that of the current standard, the microagglutination test (MA). A panel of 221 sera from 109 cases of tularemia was tested as well as 236 sera from normal individuals or individuals with other infectious or autoimmune diseases. The ICA demonstrated 91.5% ($\kappa = 0.91$) agreement with the reference method (MA) and gave an overall sensitivity of 99.3% and a specificity of 94.6%. No cross-reactivity was observed in the ICA with serum samples from normal individuals and patients with autoimmune diseases and bacterial, viral, and parasitic infections, although 4 of 50 patients with brucellosis demonstrated positive results in the ICA. The performance of ICA was simple, and it requires no specialized equipment. The ease of use and significantly high sensitivity and specificity of ICA make it a good choice for diagnostic testing and a valuable field test to support a presumptive diagnosis of tularemia in remote areas.

1. Introduction

Tularemia is a potentially fatal multisystemic disease of humans and other animals caused by the facultative intracellular bacterium *Francisella tularensis*. Tularemia occurs worldwide in the northern hemisphere between latitudes 30° and 71° north, with great variation in geographic and temporal occurrence (Sjostedt, 2007; WHO, 2007). Humans become infected through arthropod bites, handling of infected animal tissues, or ingestion of contaminated water or food, and inhalation of infectious aerosols. The clinical expression and severity of the disease in humans vary depending on the route of entrance, the virulence of the organism, and the immune status of the host. The clinical presentations of tularemia have been classically divided into 6 classic forms: ulceroglandular, glandular, oculoglandular, oropharyngeal, respiratory, and typhoidal tularemia (Ellis et al., 2002; Evans et al., 1985; WHO, 2007).

Laboratory diagnosis is usually based on direct detection of the bacteria either by culture or by nucleic acid amplification techniques and on detection of elevated antibody titers. Unequivocal laboratory diagnosis of tularemia may be accomplished by isolation of the causal agent in a clinical specimen (Splettstoesser et al., 2005; Tärnvik and Chu, 2007). Serology is the cornerstone of diagnosis in tularemia owing to the requirement of special media, prolonged incubation, and a level 3 biocontainment facility to avoid the risk of laboratory infection, as well as to the lack of standardization in polymerase chain reaction (PCR) methodology (Splettstoesser et al., 2005, 2010; Tärnvik and Chu, 2007; WHO, 2007). Antibodies to *F. tularensis* may be demonstrated by agglutination, immunofluorescence, ELISA, and Western blot (WB) (Bevanger et al., 1988; Grunow et al., 2000; Porsch-Ozcurumez et al., 2004; Sato et al., 1990; WHO, 2007). Combined IgM and IgG against *F. tularensis* can be detected by microagglutination (MA) or tube agglutination using whole killed cells. MA remains the most common method used to detect antibodies, because of its high degree of sensitivity and specificity (Splettstoesser et al., 2005, 2010; Tärnvik and Chu, 2007; WHO, 2007). However, MA and other serologic methods such as ELISA require a laboratory environment, equipment, and experienced laboratory personnel to be adequately performed.

Although the patient's exposure history may assist in narrowing the differential diagnosis, a simple, rapid, and affordable point-of-care test with high sensitivity and specificity would be useful for early diagnosis and treatment and for public health surveillance (Splettstoesser et al., 2005, 2010; WHO, 2007). An immunochromatographic assay (ICA), VIRapid Tularemia (Vircell, Granada, Spain), which detects specific IgM, IgG, and IgA antibodies against the lipopolysaccharide (LPS) of *F. tularensis*, is now available commercially. In this study, we evaluated a commercially available ICA for the qualitative

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detection of total antibodies against *F. tularensis* which can be used in laboratories with little specialized equipment.

2. Material and methods

2.1. Case sera

Specimens were selected from serum samples submitted to the National Tularemia Reference Laboratory of Turkey between 2009 and 2011. Two groups of specimens were studied. The first was composed of banked serum specimens collected from individual cases and outbreak investigations. A total of 203 sera from 106 cases were included in the group; 64 of the cases were confirmed by MA and 42 were culture confirmed. Tularemia was clinically diagnosed in oropharyngeal ($n = 103$) and oculoglandular ($n = 3$) form, which are related to the consumption of contaminated water and exposure to contaminated water by washing the face or splashes. In 42 patients in whom tularemia was clinically diagnosed in oropharyngeal form, *F. tularensis* subsp. *holarctica* had been cultured from throat culture and/or lymph node aspirates. The panel of case sera (203 specimens) consisted of 106 acute-phase sera (specimens obtained 0 to 14 days after onset of illness) and 97 convalescent-phase sera (specimens obtained 15 or more days from onset of illness). Paired sera were available for 94 cases (64 were confirmed by MA and 30 were culture confirmed) and 3 cases had more than 2 convalescent-phase specimens. Specimens from cases were collected from 4 to 42 days after the onset of illness (Table 1).

Follow-up samples from 3 patients were enrolled in this study in order to determine the reactivity of sera over time. A total of 18 sera were obtained at outpatient visits between 1 and 36 months after their episode of tularemia (i.e., late-phase sera). Among the subjects providing late-phase sera, the diagnosis of tularemia had previously been confirmed by MA and PCR analysis.

2.2. Control sera

A total of 236 control specimens were obtained; 85 were from blood donors and 151 were from individuals known to have 1 of 16 diseases other than tularemia. The serum panel was composed of banked serum specimens collected from patients with autoimmune diseases (rheumatoid factor, antinuclear antibody, and antineutrophil cytoplasmic antibody), and bacterial, viral, and parasitic infections. The number of specimens in each disease category ranged from 5 to 50 (Table 2).

2.3. Agglutination assay

MA was performed as recently described (Splettstoesser et al., 2010; WHO, 2007) using safranin-stained *F. tularensis* subsp. *holartica* live vaccine strain cells incubated overnight with serial dilution of the specimens. According to the Turkish legal health regulations, the diagnosis of tularemia is serologically confirmed by the presence of at least one of the following laboratory findings: i) the presence of compatible clinical signs or symptoms, together with the presence of specific antibodies at significantly high titers (≥1:160); or ii) a 4-fold or greater increase in 2 successive titers (the latter ≥1:160). Therefore, antibody titers of ≥1:160 were considered to be positive.

Table 1
Serum specimens from tularemia-suspected and culture-confirmed cases.

Day of obtainment	Specimen category			
	Acute-phase sera[a] ($n = 106$)		Convalescent-phase sera[b] ($n = 97$)	
	Culture negative	Culture positive	Culture negative	Culture positive
4–7	18	2		
8–10	26	10		
11–14	32	18		
15–18			30	18
19–21			11	17
22–42			14	7
Total	76	30	55	42

[a] Specimens collected within 14 days of onset of disease.
[b] Specimens collected >14 days after the onset of disease.

Table 2
Cross-reactivity of each assay with serum specimens from healthy volunteers and those previously diagnosed with other infectious or autoimmune diseases.

Condition or discharge diagnosis of patient (specimen category)	Total no. of specimens tested	No. (%) of specimens positive by	
		ICA	MA
Brucellosis	50	4 (8.0%)	3 (6.0%)
Tuberculosis (cervical lymphadenitis)	15	0	0
Cat scratch disease	8	0	0
Toxoplasmosis	12	0	0
Legionellosis	8	0	0
Epstein–Barr virus	15	0	0
HIV[a]	5	0	0
Cytomegalovirus	5	0	0
Viral hepatitis	5	0	0
Hantaviral infection	5	0	0
Lyme disease	5	0	0
Atypical pneumonia	8	0	0
Autoimmune diseases (rheumatoid factor, ANA[b], ANCA[c])	10	0	0
Blood donors	85	0	0

[a] HIV = Human immunodeficiency virus.
[b] ANA = Antinuclear antibodies.
[c] ANCA = Antineutrophil cytoplasmic antibodies.

2.4. Immunochromatographic assay

A commercially available ICA was obtained from the manufacturer (Vircell, Granada, Spain) and was used according to the manufacturer's recommendations. The ICA was manufactured using LPS extracted by the hot phenol–water method from *F. tularensis* NCTC 10857 strain cell suspension. LPS was adsorbed on both the conjugate and the test line to generate a lateral flow ICA. A control line was also included to check the correct performance of the test. The test was performed by the addition of 20 μL of serum to the sample pad of the assay device followed by the addition of running fluid. Test results were read after 15 min by visual inspection for staining of the antigen and control lines. The intensity of staining of the reaction band was read visually and scored from 1 to 3 with a color reference diagram.

2.5. Statistical analysis

The sensitivity, specificity, and related confidence intervals were calculated by using the Fisher exact test. The degree of agreement between ICA and reference assay was estimated by the kappa test (Altman, 1991).

3. Results

This study evaluated the performance of a rapid tularemia diagnostic-line assay on 203 sera from 106 confirmed cases of tularemia. ICA detected 140 of 203 sera obtained from patients with paired acute- and convalescent-phase banked serum panel specimens. ICA and MAT were positive in 41 (97.6%) of 42 culture-confirmed cases.

Of the 16 infectious or autoimmune disease groups of controls, false-positive test results were obtained for sera from 4 conditions