Available online at www.sciencedirect.com
ScienceDirect
Diagnostic Microbiology and Infectious Disease 62 (2008) 142–150
DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE
www.elsevier.com/locate/diagmicrobio

New rapid test for paratyphoid a fever: usefulness, cross-detection, and solution

Frankie C.H. Tam[a], Mingliu Wang[b], Baiqing Dong[b], Danny T.M. Leung[a], Chun Hung Ma[a], Pak Leong Lim[a,*]

[a]*Clinical Immunology Unit, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong*
[b]*Guangxi Center for Disease Prevention and Control, Nanning, Guangxi, 530028 China*

Received 1 April 2008; accepted 3 July 2008

Abstract

We described a 5-min colorimetric test for paratyphoid A fever, which detects anti-*Salmonella* O2 antibodies by inhibiting the binding between 2 types of reagent particles. This test (TUBEX-PA) is based on that (TUBEX-TF) used for typhoid fever, which detects anti-O9 antibodies. TUBEX-PA showed a sensitivity of 81.0% (47/58 culture-confirmed patients) to 93.3% (14/15) and was 98.1% (52/53) specific for healthy subjects. However, TUBEX-PA also detected 50% (7/14) to 81.8% (9/11) of typhoid patients, and conversely, TUBEX-TF detected 46.7% (7/15) to 73.3% (11/15) of paratyphoid A cases. This cross-detection could be abrogated in both tests by adding a blocker (heterologous antigen) to remove the antibodies responsible, which presumably bind to a common antigen (O12) located close to O2 and O9. The presence of anti-O12 antibodies in typhoid (9/12 or 75.0% sensitive) and paratyphoid A (22/33 or 66.7%) patients was demonstrated directly using a prototypic TUBEX test designed specifically to detect these antibodies. Thus, using TUBEX-PA and TUBEX-TF together can increase the diagnostic accuracy of detecting both typhoid and paratyphoid A fever, while the further use of differential tests allows possible immediate discrimination between these diseases.

Keywords: Rapid diagnostics; *Salmonella*; Typhoid; Antibodies; Infectious disease; Immunoassays

1. Introduction

The Widal test is a milestone invention in medicine. Devised by Frank Widal in 1896 (Widal, 1896) to diagnose typhoid fever, this test ushered in an important new science —serology or immunodiagnosis by its modern name—the practice of which is crucial to patient management nowadays. The Widal test detects the presence of serum antibodies in a patient to the causative bacterium, *Salmonella enterica* serotype Typhi (*S*. Typhi), by the simple ability of these antibodies to agglutinate a suspension of the organisms. Unknown to Widal, such agglutination tests greatly favor the detection of immunoglobulin (Ig) M antibodies over IgG antibodies, which is important because IgM antibodies are found only in, and hence diagnostic of, current infections, whereas IgG antibodies can persist long after subsidence of the disease.

S. Typhi belongs to a very large family of *Salmonella* organisms that are antigenically distinguishable from one another based on the lipopolysaccharide (LPS) somatic "O" antigens and the flagellar "H" antigens (Fig. 1A). Only 3 other members—*Salmonella* Paratyphi A, *S*. Paratyphi B, and *S*. Paratyphi C—cause a similar typhoid-like (paratyphoid) disease, whereas the other salmonellae that are usually noninvasive cause a less debilitating local infection in the gastrointestinal tract. Clinically, it is often difficult to distinguish these systemic infections from one another (Vollaard et al., 2005) or from other febrile syndromes such as dengue fever, rickettsial infections, and malaria (Bottieau et al., 2006) that frequently prevail in the same geographic regions. Based on the O antigens, the *Salmonella* organisms can be divided into several groups (Kauffmann

* Corresponding author. Clinical Immunology Unit, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong. Tel.: +852-2632-3031; fax: +852-2632-3031.
E-mail address: pllim@iggene.com (P.L. Lim).

0732-8893/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.diagmicrobio.2008.07.002

A

Group	Salmonella serotype	O Antigens	H antigens	
			Phase 1	Phase 2
A	**S. Paratyphi A**	*1*, 2, 12	a	–
B	**S. Paratyphi B**	*1*, 4, (5), 12	b	1, 2
	S. Stanley	*1*, 4, (5), 12, 27	d	1, 2
	S. Typhimurium	*1*, 4, (5), 12	i	1, 2
C1	**S. Paratyphi C**	6, 7, (Vi)	c	1, 5
	S. Choleraesuis	6, 7	c	1, 5
C2	S. Manhattan	6, 8	d	1, 5
D	S. Sendai	*1*, 9, 12	a	1, 5
	S. Typhi	9, 12, Vi	d	–
	S. Dublin	*1*, 9, 12, (Vi)	g, p	–
E1	S. Anatum	3, 10	e, h	1, 6

B

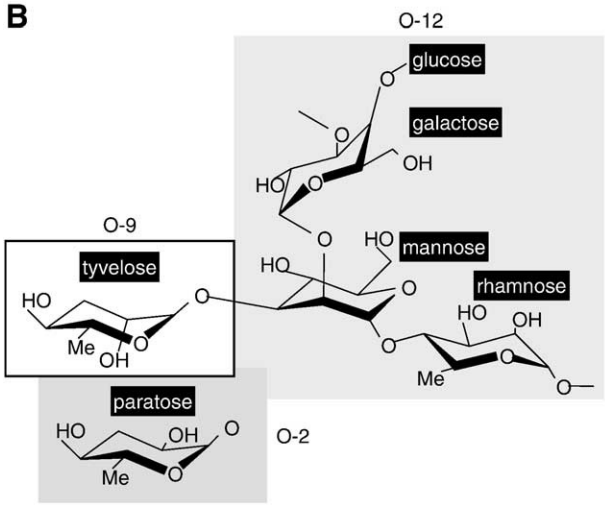

Fig. 1. The *Salmonella* antigens. (A) Grouping of *Salmonella* organisms according to the Kauffmann–White scheme based on the O and H antigens. Only a few groups and a few members in each are shown. The group-specific O antigens and the organisms causing the typhoid and paratyphoid fevers are indicated by bold print. (B) Chemical structure of the O9 or O2 antigen linked directly to the O12 antigen as a repeating unit of the LPS structure in the cell wall of *Salmonella* bacteria (adapted from Steinbacher et al., 1996.)

and Edwards, 1957). Thus, *S.* Typhi belongs to serogroup D in which the group-specific and immunodominant antigen is called O9. This is a terminal epitope composed essentially of tyvelose, a very unique sugar in nature, which is linked directly to a trisaccharide repeating unit in the LPS structure (mannose–rhamnose–galactose) (Jann and Westphal, 1975) that itself specifies the O12 antigen (Fig. 1B). O12 is also found in *S.* Paratyphi A (serogroup A) but is, in this case, adjoined to paratose that forms the O2 antigen, the counterpart of O9 (Fig. 1B). The 2 LPSs are otherwise identical to each other. The LPS structure of *S.* Paratyphi B and *S.* Typhimurium, both belonging to serogroup B, is also very similar to these, including the presence of O12, except for the immunodominant antigen (O4 in this case; Fig. 1A). Like typhoid, paratyphoid A fever has been commonly diagnosed by culture and the Widal test based on the O and H antigens using, in this case, *S.* Paratyphi A organisms.

Typhoid fever remains a global health problem. In 2000, it affected an estimated 21.7 million people worldwide, especially those living in South and Southeast Asia (>100 cases per 100 000 people per year), including Africa, Latin America, the Caribbean as well as parts of Oceania and Asia (10–100 cases per 100 00 0 per year), causing an estimated 216 500 deaths in all (Crump et al., 2004). The paratyphoid fevers, of which paratyphoid A is usually the most severe and common, affected some 5.4 million people in the same year. The Widal test has continued to be widely used in the endemic countries despite serious shortcomings (Chart et al., 1998; Nsutebu et al., 2002; Parry, 2004) and the availability of more objective and sophisticated tests such as those based on ELISA (House et al., 2001; Nardiello et al., 1984).

The popularity of the Widal test may be attributed to the simplicity (1-step procedure) and user-friendliness of the test. The latter is part of a greater number of attributes (abbreviated "ASSURED") that are presently recognized as desirable by a diagnostic test for developing countries (Mabey et al., 2004). Other attributes require the test to be affordable and deliverable, sensitive and specific, as well as rapid and instrument free, that is, useable as a point-of-care application. A rapid test introduced recently for typhoid fever that meets many of these criteria is TUBEX-TF (IDL Biotech, Borlange, Sweden). This test is most Widal like in characteristics compared with other rapid tests available currently, including those based on immunodot analysis (e.g., TyphiDot; Malaysian Biodiagnostic Research, Bangi, Malaysia), immunochromatography (e.g., SD Bioline Typhoid; Standard Diagnostics, Kyonggi-do, Korea), or ELISA (e.g., Mega Salmonella; Mega Diagnostics, Los Angeles, CA). Thus, like Widal, TUBEX-TF is simple, uses a visual readout that requires no instrumentation, and, importantly, also detects *Salmonella* O-specific antibodies in an agglutination-type assay. IgM antibodies, too, are preferentially detected from whole sera (Tam and Lim, 2003), which is enhanced by IgG antibodies (Rahman et al., 2007). However, significant improvement has been made in TUBEX-TF in terms of test design and performance in several ways: 1) use of purified *S.* Typhi LPS instead of whole bacteria, which is coated onto magnetic microspheres (LPS particles) to allow rapid (magnetic) separation of reactants to hasten the test procedure to a few minutes; 2) use of a monoclonal anti-O9 antibody, which is coated onto blue-colored microspheres (indicator particles) that will bind to the LPS particles when mixed together. Binding is revealed when the mixture of particles contained in specially designed V-shaped reaction wells is placed on a magnet, which sediments not only the LPS particles but also the indicator particles associated with these. This leaves behind a clear supernatant (artificially colored red in the test) if all particles are, thus, removed. On the other hand, unbound indicator particles, if left suspended, will produce a bluish suspension, the color intensity depending on the concentration of the particles. This, in turn, depends on the amount of anti-O9 antibodies present in a patient's serum that is able to inhibit