

### Stained Agglutinating Suspensions “Febrile Antigens”

#### Introduction

Stained bacterial antigen suspensions, sometimes called “Febrile Antigens” or “Widal reagents”, are used for the identification and quantitative determination of specific antibodies in human sera, following infection with certain infectious diseases such as Enteric fevers, Brucellosis and Rickettsial infections.

These diagnostic tests were devised by early bacteriologists whose names have been given to the assays. In 1896, Max von Gruber and Herbert Edward Durham first discovered bacterial agglutination when they observed that sera from patients with typhoid fever agglutinated with the causative organism. Also in 1896, **Widal** developed a procedure for diagnosing typhoid fever based on the same principle (the Widal reaction). **Wright & Smith** (1897) then applied this technique to the diagnosis of Brucellosis. Later **Weil & Felix** (1916) described the diagnosis of Rickettsial infections using particular strains of *Proteus* spp. that have antigens in common.

#### The Agglutinating Suspension

Biotec Laboratories’ range of febrile antigens (stained bacterial suspensions) are standardised smooth suspensions of killed bacteria which have been vitally stained to aid the reading of the agglutination reaction. Blue stained antigen are specific to somatic ‘O’ antigens, whilst red stained antigens are specific to flagella ‘H’ antigens.

Febrile antigens are sensitive to heat and cold, and must be stored at 2-8°C ( $\pm 2^\circ\text{C}$ ). **Freezing will destroy the suspension.** Full details for the storage of these reagents are given in the instructions for use (IFU) provided with each product.

#### The Widal Test

This diagnostic technique was devised by Widal to detect antibodies in serum of patients suffering from typhoid fever. The use of a variety of *Salmonella* O and H antigens has allowed the application of this method to be extended to include *Salmonella* other than *S. typhi*.

The Widal test may be performed using two methods, which apply to all febrile antigens within the Biotec range (always refer to the IFU provided with the product). Firstly the ‘Rapid Slide Titration Method’, in which the febrile antigen is mixed with patient’s serum on a slide at a range of dilutions, to identify an approximate titre for the test serum. Secondly, the ‘Tube Agglutination Test Method’, in which a serial dilution of patient serum is prepared in tubes and the febrile antigen is added, followed by incubation and observation for agglutination. This method is performed to confirm the titre obtained in the slide method. **It is very important that all dilutions of the rapid slide method are performed rather than just performing a screening test at one dilution. This is to prevent false negative results due to the ‘prozone’ effect, where a high serum concentration may give a negative result, but further dilutions of the same serum give a positive result.** Vaccination against typhoid and paratyphoid fever, using killed suspensions of *Salmonella* can also produce detectable antibodies.

Although the technique is simple, interpretation can be fraught with difficulty. The highest dilution showing an agglutination reaction is termed the ‘titre’. In most cases, the antibody titre will be low during the acute phase of infection, rising during the convalescent phase (or as the vaccine becomes effective) and will eventually fall, either disappearing altogether or leaving a small residual titre (see figure 1).

**It must be emphasised that a single measure of antibody titre does not indicate active infection. Many antibodies remain at a high titre for long periods of time after infection. To establish that an acute illness is due to a particular agent, it is essential to show a rise in antibody titre in samples of serum from the same patient, taken several days apart. A four-fold rise in titre is often considered a clinically significant result.**



**Figure 1: The Course of Infection in a Typical Untreated Typhoid Fever Patient.**

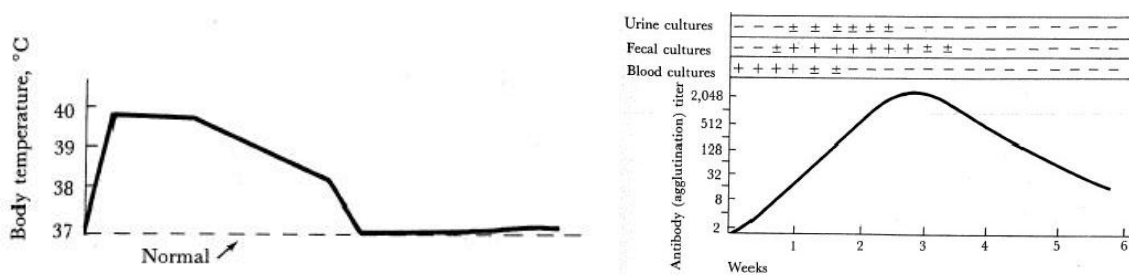


Figure 1: Measurement of body temperature provides a measure of the course of clinical symptoms. The antibody titre was measured by determining the highest dilution (two-fold series) causing agglutination of a test strain of *S. typhi*. Presence of viable bacteria in blood faeces and urine was determined from periodic cultures. Note that the pathogen clears from the blood as the antibody titre rises whereas clearance from the faeces and urine requires longer time. Body temperature gradually drops to normal as the antibody titre rises. The data given does not represent a single patient, but are a composite of the picture seen in large numbers of patients.

## Typhoid & Paratyphoid Enteric Fevers

These conditions are caused by *S. typhi* and *S. paratyphi* (*A*, *B* or *C*) respectively. The World Health Organisation estimates the annual worldwide incidence of typhoid fever to be approx. 17 million cases, with 600 000 associated deaths. Paratyphoid symptoms are similar to typhoid, but generally milder. After an incubation period of usually between 8 and 14 days, symptoms may arise including fever (as high as 39-40°C), malaise and constipation or diarrhoea. Typhoid fever is a much more serious infection and can also result in intestinal perforation and haemorrhaging.

Using a febrile antigen, O antibodies usually show positive on days 6-8 and H antibodies on days 10-12 after the onset of disease. Titres will continue to rise for some time after that. It is acknowledged that interpretation of results may be difficult because of residual titre, or what colloquially is called “background noise”, derived from previous infections or vaccinations. It is therefore important to establish the antibody level in the normal population in a particular locality in order to determine a threshold above which the titre is considered significant. Limitations of the test are detailed on the IFU.

Some *Salmonella* also have an envelope antigen called ‘Vi’ (virulence). Strains of *S. typhi* and *S. paratyphi C* (and some *Citrobacter* spp.) may possess Vi antigen that render the strains non-agglutinable in O antisera. These cultures agglutinate in Vi antiserum. They will, however, agglutinate in O antiserum after destruction of the Vi antigen.

The antigenic structure of the causative *Salmonella* for typhoid and enteric fevers is described in Table 1.

**Table 1: The Antigenic Structure of the Causative *Salmonella* for Typhoid and Enteric Fevers**

Serotype	O antigen	H antigen Phase 1:2	Serogroup
<b>Typhoid fever</b>			
<i>S. typhi</i>	IX, XII, (Vi)	d:	Group D1
<b>Paratyphoid Fever</b>			
<i>S. paratyphi A</i>	I, II, XII	a: (1, 5)	Group A
<i>S. paratyphi B</i>	I, IX, (V), XII	b: 1, 2	Group B
<i>S. paratyphi C</i>	VI, VII, (Vi)	c: 1, 5	Group C1

Antigens in parentheses are either weak or absent in some isolates.

## Brucellosis

These diseases caused by *Brucella* bacteria are characterised by “undulant fever”, a fever occurring in waves with a variable incubation time between ten and thirty days. Symptoms are commonly fever, muscular pains, drenching sweats, chills and rigors. The infection can lead to disability or even death. Another manifestation of the disease is abortion of the foetus, in animals or humans.

Various *Brucella* species affect sheep, goats, cattle and many other animals. The preferred host of *Brucella melitensis* is sheep or goat, and cattle for *Brucella abortus*. Once wild animals become infected they become the reservoir for contamination of domestic animals. Humans become infected by contact with animals or animal products, such as milk contaminated with these bacteria.

*Brucella* are Gram negative, non-motile organisms that do not possess obvious virulence factors such as a capsule or flagella. Only “O” agglutination suspensions are therefore used. *Brucella* are very small bacteria, and the agglutination reaction can be subject to the “prozone” phenomenon. For this reason it is essential to show a rise in antibody titre in samples of serum from the same patient taken several days apart. Antibodies are usually identified by the end of the second week of the disease, rising to titres of 1/640 or more. After an acute attack, the titre will fall rapidly, sinking to a low level after three or so months. However with a chronic condition the titre may fluctuate, making the organism difficult to demonstrate except in the spleen or bone marrow.

## Rickettsial Diseases

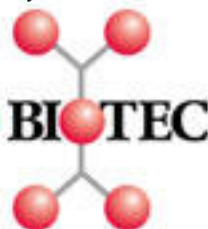
These include Typhus fever, Spotted fever, Rocky Mountain Spotted fever, Trench fever, Q fever and Scrub fever. Rickettsial diseases are caused by tiny micro-organisms which are intracellular parasites. The normal vector for disease spread is via insects or lice. The best-known Rickettsial pathogens are the causative agents of the typhus group of diseases. *Rickettsia prowazekii* is responsible for typhus fever and *Rickettsia typhi* of a similar, but less severe illness.

Diagnosis of Rickettsial infections can be made by isolation and identification of the causative agent, coupled with the demonstration of a rising antibody titre. The use of febrile antigens to detect a rising antibody titre to *Rickettsia* species is known as the **Weil-Felix** reaction, named after the workers who discovered a cross reaction between the agglutinins produced by *Rickettsia prowazekii* and certain *Proteus vulgaris* strains. Weil and Felix designated these organisms *Proteus* OX2 and *Proteus* OX19. This reaction was expanded in 1923 when Kingsbury isolated another *Proteus* strain, OXK, which gave specific reaction with serum from patients convalescing from scrub typhus.

**Note:** the Weil-Felix reaction is non-specific, so in some cases there will be no demonstrable titre to *Proteus* antigens. Conversely, patients with *Proteus* infections may show significant titres. Clearly, a definitive differentiation between species of *Rickettsia* is also not possible when using this screening test. The results obtained must therefore be correlated and interpreted with clinical findings.

## References

- Brock T. D. *et. al.*, (1984). *Biology of Microorganisms*. 4<sup>th</sup> Ed. Prentice-Hall Inc.
- Brucellosis. [www.cdc.gov/ncidod/dbmd/diseasinfo/brucellosis\\_g.htm](http://www.cdc.gov/ncidod/dbmd/diseasinfo/brucellosis_g.htm).
- Rickettsial Diseases, *The Merck Manual of Diagnosis and Therapy*. [www.merck.com/mrkshared/mmanual/section13/chapter159/159a.jsp](http://www.merck.com/mrkshared/mmanual/section13/chapter159/159a.jsp).
- World Health Organisation. Background document: The diagnosis, treatment and prevention of typhoid fever. *Communicable Disease Surveillance and Response, Vaccines and Biologicals*. WHO/V&B/03.07.
- World Health Organisation. *Water-related Diseases*, [www.who.int/water\\_sanitation\\_health/diseases/typhoid/en/](http://www.who.int/water_sanitation_health/diseases/typhoid/en/).



# BIOTEC Febrile Antigens

## Febrile Antigens

Listed below are the febrile antigen products available from Biotec Laboratories Ltd. Please contact your local Biotec distributor for further details.

<u>Cat No:</u>	<u>Description:</u>	<u>Size:</u>
2/002	Brucella Abortus Antigen	5ml
2/004	Brucella Melitensis Antigen	5ml
2/006	Proteus OXK Antigen	5ml
2/008	Proteus OX2 Antigen	5ml
2/010	Proteus OX19 Antigen	5ml
2/012	Salmonella O Antigen Group A	5ml
2/014	Salmonella O Antigen Group B	5ml
2/016	Salmonella O Antigen Group C	5ml
2/018	Salmonella O Antigen Group D (Typhi O)	5ml
2/022	Salmonella H Antigen Group a	5ml
2/024	Salmonella H Antigen Group b	5ml
2/026	Salmonella H Antigen Group c	5ml
2/028	Salmonella H Antigen Group d (Typhi H)	5ml
2/030	Febrile positive control sera	2.5ml
2/032	Febrile negative control sera	2.5ml
2/034	Febrile antigen kit comprising 8 specified antigens	8x5ml
2/040	Febrile antigen kit comprising 8 specified antigens	8x5ml
	with positive and negative controls	2x2.5ml
2/050	Salmonella Vi	5ml

