SALMONELLA SEROLOGY

SEROLOGICAL DIAGNOSTIC OF TYPHOID AND PARATYPHOID FEVERS

IVD CE

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BIO-RAD

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1- CLINICAL VALUE

Salmonella are Enterobacteriaceae that infest the gastrointestinal tract of man and animals, causing major (typhoid or paratyphoid fever) or minor salmonellosis (gastroenteritis). Although the presence of anti-*Salmonella* antibodies may raise the suspicion of recent infection, the definitive diagnosis can only be established by isolation of the pathogen.

2- PRINCIPLE

The Widal and Felix serological diagnosis of typhoid and paratyphoid fevers is a technique for detection and assay of specific anti-O and anti-H agglutinins by dilution of the patient's serum. H agglutination is rapid (2 hours) and flocculent at 37°C, while O agglutination occurs more slowly (18 hours) and is granular at room temperature (18-30°C). A positive result is interpreted by comparing anti-O and anti-H agglutinin titres on one or several samples.

Remark : Although *S. paratyphi C infections* are very rare in Europ, CO and CH suspensions are prepared since these can be used to identify infections due to those *Salmonella* strains which carry the same O and H antigens as this serotype. *S. paratyphi* A infections are rare in France, but are frequently found in the Far East and in Africa.

3- PRODUCT INFORMATION

- **Presentation :** 1 vial of 50 ml.

Label	Code
Salmonella typhi Antigen O (TO)	63402
Salmonella typhi Antigen H (TH)	63312
Salmonella typhi Antigen Vi	63572
Salmonella paratyphi A Antigen O (AO)	63412
Salmonella paratyphi A Antigen H (AH)	63322
Salmonella paratyphi B Antigen O (BO)	63422
Salmonella paratyphi B Antigen H (BH)	63332
Salmonella paratyphi C Antigen O (CO)	63432
Salmonella paratyphi C Antigen H (CH)	63342
Salmonella typhimurium Antigen H (TMH)	63542
Salmonella enteritidis Antigen H (ENH)	63272

- **Storage** : at + 2-8 °C.
- **Shelf-life** : until the expiry date printed on the package (even once opened).

4- PRECAUTIONS

The reliability of the results depends on correct implementation of the following Good Laboratory Practices:

- Before use, allow reagents to reach room temperature (+18 - 30°C).
- Do not use expired reagents.
- Use glassware washed and rinsed with distilled water or preferably disposable material.
- Use a new pipette tip for each sample.

Health and safety instructions

- Wear disposable gloves when handling reagents.
- Do not pipette by mouth.
- Any material that comes directly in contact with samples, should be considered as if capable of transmitting infectious disease.
- Avoid samples from spilling.
- For hazard and precaution recommendations related to some chemical components in this test kit, please refer to the pictogram(s) mentioned on the labels and the information supplied at the end of instruction for use. The Safety Data Sheet is available on www.bio-rad.com.

5- SPECIMEN

1. Serum collected on dry tubes is the recommended sample type.
2. Observe the following recommendations for handling, processing, and storing serum samples:
   - Collect all serum samples observing routine precautions.
   - Allow samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - After centrifugation separate the serum and store it in a tightly stoppered storage tube.
   - The specimens can be stored at +2- 8°C if screening is performed within 24 hours.
   - If the assay will not be completed within 24 hours, or for shipment of samples, freeze at –20°C, or colder.
   - Preferably, thaw samples once only. Previously frozen specimens should be thoroughly mixed after thawing prior to testing.

3. Interferences due to high levels of albumin or bilirubin are unknown. Do not use lipemic or hemolyzed samples.
4. Do not heat the samples.

6- PROCEDURE

A) MATERIALS REQUIRED NOT PROVIDED

- Physiological water.
- Hemolysis tubes.
- Incubator or water-bath
- Disposable gloves
- Automatic or semi-automatic, adjustable or preset, pipettes or multipipettes to measure and dispense 10 to 1000 µl and 1, 2 and 10 mL.

B) CLASSICAL TECHNIQUE WITH INCUBATION AT 37°C

1. Make 1/10, 1/20, 1/40, 1/80 and 1/160 dilutions of serum in physiological water.
2. For each suspension, add to a series of tubes:
   - 0.1 ml of each serum dilution
   - 0.9 ml suspension

In this way, the final dilutions of serum obtained range from 1/100 to 1/1.600 for each suspension.

3. Incubate in a 37°C water-bath or incubator, for 2 hours.
4. At the end of the incubation period, examine the tubes containing H suspension : H agglutination, which is flaky and easily dissociable (do not shake the tubes violently), may be observed if the serum contains H agglutinins corresponding to one of the suspensions.
5. The tubes containing O suspensions are left at room temperature (18-30°C) until the next day.
6. After this time, O agglutination may be observed. It is fine, granular and difficult to dissociate. A concave mirror may facilitate the examination of the tubes.

C) RAPID CENTRIFUGATION TECHNIQUE

1. Make two dilutions of the sera:
   - Serum 0,2 0,1
   - Physiological saline 1,8 1,9
   - Dilution 1/10 1/20
2. Place 4 rows of 4 unflanged haemolysis tubes in a test tube rack.

Tube N°	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Serum 1/20		0.1		0.1		0.1		0.1		0.1		0.1		0.1		0.1
Serum 1/10	0.1		0.1		0.1		0.1		0.1		0.1		0.1		0.1	
Add to each tube 0.9 ml of suspensions	TO	TO	TH	TH	OA	OA	AH	AH	BO	BO	BH	BH	CO	CO	CH	CH
Final dilution	1/100	1/200	1/100	1/200	1/100	1/200	1/100	1/200	1/100	1/200	1/100	1/200	1/100	1/200	1/100	1/200

3. Centrifuge at about 3000 r.p.m. for 5 minutes (until the bacteria have sedimented).
4. Resuspend the precipitate by tapping the bottom of the tube. If the suspension becomes homogeneous, the result is negative. A positive result is indicated by the presence of agglutination visible with the naked eye.

If, for example, agglutination is observed with TO and TH suspensions at a dilution of 1/200, while the rest remains negative, the test must be repeated with 1/40, 1/80 and 1/160 dilutions of serum so as to measure the titre of agglutinins in the serum. This is only carried out for those suspensions where agglutination has occurred (in this case, TO and TH). Final titres will be 1/400, 1/800 and 1/1600.

Remark : the morphology of the agglutinates is less differentiated than with the classic method.

7- INTERPRETATION OF RESULTS

- Anti-O agglutinins appear on the 8th day in typhoid or paratyphoid fevers, reach a mean titre of 1/400 and then rapidly disappear after clinical cure. Anti-H agglutinins appear between the 10th and 12th days, reach a mean titre of 1/800 to 1/1600, decline over the weeks following clinical cure and persist for months or even years at a mean titre of 1/100 to 1/200.
- Injection of a TAB vaccine induces production of low titres of anti-O and anti-H agglutinins. Anti-H agglutinins sometimes persist for T, B and sometimes A at titres of less than 1/200.
- If a patient with typhoid fever has previously been treated with potent antibiotic, O agglutinins, which are normally indicative of the disease, may not appear. Diagnosis can only be based on a significant increase in H agglutinin titre in two samples taken several days apart.

Exemple : if only TO agglutinins are found, it is probably at an early stage of *S. typhi* typhoid fever; a second agglutination test carried out a few days later will show up the TH agglutinins.

If, in this case, an anti-H agglutinin, other than anti-TH, is detected, the infection may also be caused by a *Salmonella* strain carrying an O antigen similar to that of *S. typhi*; the most common strain of this type is *S. enteritidis*. Agglutination with a *S. enteritidis* H suspension should be included to test this possibility.

If only BO suspension is agglutinated, proceed as above using an H suspension of *S. typhimurium*.

8- VI SEROLOGICAL DIAGNOSTIC

A) SUSPENSION

The antigenic suspension is prepared from *S. typhi* not agglutinated by O antiserum, treated with alcohol (destruction of the H antigen) and with 5 g/l $CaCl_2$ added to stabilise the vi antigen.

B) METHOD

As for the detection of O agglutinins, but using final dilutions of 1/10, 1/20 and 1/40…

C) RESULTS

Vi antibodies appear at a later stage of the illness than O and H antibodies. It is useful to detect the healthy Eberth bacillus carriers in epidemiological studies. However, isolation of *S. typhi* is the only way in which carriers may be definitely identified.

9- PERFORMANCES / QUALITY CONTROL OF THE TEST

Performances of *Salmonella* Serology kits are controlled with Antiserum *Salmonella* polyvalent T, A, B, C, Vi (code 61261), Antiserum *Salmonella* monovalent H: i (code 60161) and Antiserum *Salmonella* monovalent H:g,m (code 61121) as follow :

Dilute agglutinating serum in physiological water 1/100 for anti-H agglutinines detection, 1/50 for anti-O agglutinines detection and 1/10 for anti-Vi agglutinines detection. For incubation and reading, follow instructions for classical technique. Observe corresponding agglutinations.

10- QUALITY CONTROL OF THE MANUFACTURER

All manufactured reagents are prepared according to our Quality System, starting from reception of raw material to the final commercialization of the product. Each lot is submitted to quality control assessments and is only released to the market, after conforming to pre-defined acceptance criteria. The records relating to production and control of each single lot are kept within Bio-Rad.

(BG) • Този продукт съдържа човешки или животински компоненти. Бъдете внимателни при работа с него.

(CZ) • Tento výrobek obsahuje lidské nebo zvířecí komponenty. Zacházejte s ním opatrně.

(DE) • Dieses Produkt enthält Bestandteile menschlichen oder tierischen Ursprungs. Vorsichtig handhaben.

(DK) • Dette produkt indeholder humane og animalske komponenter. Skal behandles med forsigtighed.

(EE) • Käesolev toode sisaldab inim-või loomseid komponente. Käsitseda ettevaatlikult.

(EN) • This product contains human or animal components. Handle with care.

(ES) • Este producto contiene componentes humanos o animales. Manejar con cuidado.

(FI) • Tässä tuotteessa on ihmisestä tai eläimistä peräisin olevia osia. Käsittele varovasti.

(FR) • Ce produit contient des composants d'origine humaine ou animale. Manipuler avec précaution.

(GR) • Αυτό το προϊόν περιέχει ανθρώπινα ή ζωικά στοιχεία. Χειριστείτε το με προσοχή.

(HR) • Ovaj proizvod sadrži ljudske ili životinjske sastojke. Pažljivo rukovati.

(HU) • A készítmény emberi vagy állati eredetű összetevőket tartalmaz. Óvatosan kezelendő.

(IT) • Questo prodotto contiene componenti umane o animali. Maneggiare con cura.

(LT) • Šiame produkte yra žmogiškosios arba gyvūninės kilmės sudėtinių dalių. Elgtis atsargiai.

(NL) • Dit product bevat menselijke of dierlijke bestanddelen. Breekbaar.

(NO) • Dette produktet inneholder humane eller animalske komponenter. Håndteres med forsiktighet.

(PL) • Niniejszy produkt zawiera składniki pochodzenia ludzkiego lub zwierzęcego. Należy obchodzić się z nim ostrożnie.

(PT) • Este medicamento contém componentes de origem humana ou animal. Manuseie com cuidado.

(RO) • Acest produs conține materiale de origine umană sau animală. Manevrați-l cu grijă.

(SE) • Denna produkt innehåller beståndsdelar från människa eller djur. Hantera produkten varsamt.

(SI) • Izdelek vsebuje človeške ali živalske sestavine. Rokujte previdno.

(SK) • Tento výrobok obsahuje ľudské alebo zvieracie zložky. Narábajte s ním opatrne.

H333

(BG)
внимание
Може да причини вреда при вдишване.

(CZ)
Varování
Může škodit zdraví při vdechnutí.

(DE)
Achtung
Kann beim Einatmen gesundheitsschädlich sein.

(DK)
Advarsel
Kan være sundhedsskadelig ved indånding.

(EE)
Hoiatus
Kahjulik sissehingamisel.

(EN)
Warning
May be harmful if inhaled.

(ES)
Atención
Puede ser nocivo si se inhala.

(FI)
Varoitus
Mahdollisesti haitallinen hengitettynä.

(FR)
Attention
Peut être nocif par inhalation.

(GR)
Προσοχή
Πιθανά βλαβερό κατά την εισπνοή.

(HR)
Upozorenje
Može Štetno ako se udiše.

(HU)
Figyelem
Belélegzés esetén veszélyes lehet.

(IT)
Attenzione
Può essere nocivo per inalazione.

(LT)
Atsargiai
Gali būti kenksminga įkvėpus.

(NL)
Waarschuwing
Kan schadelijk zijn bij inademing.

(NO)
Advarsel
Kan være helsefarlig ved innånding.

(PL)
Uwaga
Może działać szkodliwie przez drogi oddechowe.

(PT)
Atenção
Pode ser nocivo em caso de inalação.

(RO)
Atenţie
Poate fi nociv dacă este inhalat.

(SE)
Varning
Kan vara skadlig att inandas.

(SI)
Pozor
Možna nevarnost trajnih okvar zdravja pri vdihavanju.

(SK)
Pozor
Môže byť škodlivé pri nadýchaní.

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