Website : www.erbamannheim.com

ERBA T-LTX

CRP

CE IVD

INTENDED USE
Quantitative determination of C reactive protein (CRP), in human serum or plasma by Latex-enhanced turbidimetric immunoassay

CLINICAL SIGNIFICANCE
CRP is an acute-phase protein present in normal serum,which increases significantly after most forms of tissue injuries,bacterial and virus infections,inflammation and malignant neoplasia. During tissue necrosis and inflammation resulting from microbial infections, the CRP concentration can rise up to 300 mg/L in 12-24 hours

METHODOLOGY
Quantitative turbidimetric immunoassay.

PRINCIPLE
Latex particles coated with specific anti- human CRP are agglutinated when mixed with samples containing CRP. The agglutination causes an absorbance change, dependent upon the CRP contents of the patient sample that can be quantified by comparison from a calibrator of known CRP concentration.

REAGENT COMPOSITION
DILUENT (R1) : Tris buffer 20 mmol/L, pH 8.2
LATEX (R2) . Latex particles coated with goal IgG anti-human CRP, pH 7.3 with Preservative.
CRP-CAL : Calibrator - C-Reactive protein concentration is stated on the vial label

PREPARATION
CRP Calibrator: Reconstitute with 1.0 mL of DI water. Mix gently and incubate 10 minutes at room temperature before use.

STORAGE AND STABILITY
All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C and contaminations are prevented during their use Do not use reagents over the expiration date.
Reagent Deterioration: The CRP latex reagent should have a white, turbid appearance free of granular particulate. Visible agglutination or precipitation may be a sign of deterioration and the reagent should be discarded
The CRP buffer reagent should be clear and colourless. Any turbidity may be sign of deteroration and the reagent should be discarded.
CRP Calibrator: Stable for 1month at 2-8°C or 3months at -20°C.
Diluent & Latex reagent.:
Do not freeze; frozen Latex or Diluent could change the functionality of the test

SAMPLES
Fresh serum. Stable 7 days at 2-8°C or 3 months at –20°C.
The samples with presence of fibrin should be centrifuged before testing.
Do not use highly hemolized or lipemic samples.

ASSAY PARAMETERS

Mode	Two Point / Fixed Time
Wavelength (nm)	546
Sample Volume (µl)	10
Reagent Volume (µl)	800µl R1 + 200µl R2
Lag Time (Sec.)	5
Kinetic Interval (Sec.)	120
No. of readings	1
Reaction Temperature (°C)	37°C
Reaction Direction	Increasing
Normal Low (mg/L)	0
Normal High (mg/L)	6
Linearity Low (mg/L)	2
Linearity High (mg/L)	80
Absorbance Limit (Max)	NA
Blank with	DI Water
Calibrator Concentration (mg/L)	Refer to Vial
Units	mg/L

Programme parameters for specific clinical analysers are available on request.

ASSAY PROCEDURE

Pipette into tube	Blank	Test
Reagent - 1	800µl	800µl
Reagent - 2	200µl	200µl
Sample / Calibrator	--	10 µl

Mix well and aspirate.

REFERENCE VALUES
Normal values up to 6 mg/L or 0.6 mg/dL.
Each laboratory should establish its own reference range.

UNIT CONVERSION
mg/L = mg/dL x 10

INTERFERENCES
Bilirubin (20 mg/dL), lipemia (10 g/L) and rheumatoid factors (300 IU/mL) do not interfere. Hemoglobin (≥5 g/L),interferes. Other substances may interfere as mentioned in Young DS.

Manufactured by : **TRANSASIA BIO-MEDICALS LTD.,** Khatiyan No. 235, Khasra No.24, Namthang Elaka. Mahakuma Namchi, South Sikkim – 73713
In Technical Collaboration with : ERBA diagnostics Mannheim GmbH **Mallaustr., 69-73, D - 68219, Mannheim / Germany**

R, 400
R 100
S S

CALCULATION

ΔA = A2-A1

$$\text{CRP (mg/L)} = \frac{\Delta\text{Abs of Test}}{\Delta\text{Abs of Cal}} \times \text{Conc of Cal. (mg/L)}$$

PERFORMANCE CHARACTERISTICS

1. Linearity limit: Up to 80 mg/L under the described assay conditions. Samples with higher concentrations should be diluted 1/5 in NaCl 9 g/L and retested again. The linearity limit depends on the sample / reagent ratio, as well as the analyzer used. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.

2. Detection limit: Values less than 2 mg/L give non-reproducible results.

3. Prozone effect: No prozone effect was detected upon 800 mg/L.

4. Sensitivity: Δ4.2 mA mg/L.

5. Precision: The reagent has been tested for 20 days, using three different CRP concentrations in a EP5-based study.

EP5	CV (%)		
	9.2 mg/L	16.8 mg/L	57.97 mg/L
Total	7.30%	6.90%	5.90%
Within Run	2.80%	3.10%	2.90%
Between Run	6.10%	4.70%	3.90%
Between Day	3.00%	4.00%	3.40%

SYMBOLS:

The following symbols are used in the labeling of ERBA Mannheim kits

REF	Catalogue No	CE	CE Mark - Device comply with the Directive 98/79/EC
LOT	Batch Code	IVD	In Vitro Diagnostics
	Expiry Date (Last day of the month)		Consult Instruction for Use
	Manufactured by		Storage temperature
CRP	Product Name	CONT	Content

6. Accuracy: Results obtained using this reagent (y) were compared to those obtained using a commercial reagent (x) with similar characteristics. 50 samples of different concentrations of CRP were assayed. The correlation coefficient (r) was 0.99 and the regression equation y = 1.101x + 2.518.
The results of the performance characteristics depend on the analyzer used.

PRECAUTIONS

1. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

2. Components from human origin have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). However handle cautiously as potentially infectious.

WASTE MANAGEMENT

As per local legal requirements

REFERENCES

1. Lars-Olof Hanson et al. Current Opinion in Infect Diseases 1997, 10 196-201.
2. Chetana Vaishnavi. Immunology and Infectious Diseases 1996, 6 139 – 144.
3. Yoshitsugy Hokama et al. Journal of Clinical Lab. Status 1987; 1: 15 – 27.
4. Kari Pulki et al. Sacand J Clin Lab Invest 1986, 46: 606 – 607.
5. Werner Muller et al. Journal of Immunological Methods 1985; 80: 77 – 90.
6. Shogo Otsuji et al. Clin Chem 1982; 28/10: 2121 – 2124.
7. Young DS. Effects of drugs on clinical laboratory test, 4th ed. AACC Press, 1995.

PACK PRESENTATION

Product code	Pack size	Reagent-1	Reagent-2	Calibrator
131959	1 x 40, 1 x 10, 1x 1ml	1 x 40ml	1 x 10ml	1 x 1ml

Revision No.: 0- CRP
Date of Issue : 01/11/2019

ISO 9001, ISO 13485
QUALITY SYSTEM CERTIFIED

HEPACARD

One Step Rapid Visual Test For the Qualitative Detection of HBsAg in Human Serum/Plasma

INTENDED USE

HEPACARD is a visual, rapid, sensitive and accurate one step immunoassay for the qualitative detection of Hepatitis B Surface Antigen (HBsAg) in Human Serum or Plasma. The assay is intended to be used as an aid in the recognition and diagnosis of acute infections and chronic infectious carriers of the Hepatitis B Virus (HBV).

INTRODUCTION

The antigenic determinant of the HBsAg protein moiety is antigenically heterogenous and it determines specific HBV serotypes and provides a basis for immunodetection. The principal antigenic determinant is "a" which is common to all HBV serotypes. In addition, two pairs of subspecific determinants have been identified, d/y & w/r, which are apparently mutually exclusive. Four antigenic combinations are therefore possible: adw, adr, ayw and ayr.

PRINCIPLE

HEPACARD is a one step immunoassay based on the antigen capture, or "sandwich" principle. The method uses monoclonal antibodies conjugated to colloidal gold and polyclonal antibodies immobilized on a nitrocellulose strip in a thin line. The test sample is introduced to and flows laterally through an absorbent pad where it mixes with the signal reagent. If the sample contains HBsAg, the colloidal gold-antibody conjugate binds to the antigen, forming an antigen-antibody-colloidal gold complex. The complex then migrates through the nitrocellulose strip by capillary action. When the complex meets the line of immobilized antibody (Test line) "T", the complex is trapped forming an antibody-antigen-antibody colloidal gold complex. This forms a pink band indicating the sample is reactive for HBsAg. To serve as a procedural control, an additional line of anti-mouse antibody (Control line) "C", has been immobilized at a distance from the test line on the strip. If the test is performed correctly, this will result in the formation of a pink band upon contact with the conjugate.

KIT CONTENTS

a) Hepacard Test Device

b) Sample Dropper

c) Instruction Manual

KIT PRESENTATION

100 Test Pack

200 Test Pack

STORAGE AND SHELF LIFE

HEPACARD should be stored at 2-30°C in the coolest and driest area available. Expiry date on the kit indicates the date beyond which the kit should not be used. **The HEPACARD should not be frozen and must be protected from exposure to humidity.**

WARNING FOR USERS

⚠ **CAUTION:** ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for *in vitro* diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. All materials used in the assay and samples should be decontaminated in suitable disinfectant solution for 30-60 min before disposal or by autoclaving at 121°C at 15psi for 60 min. They should be disposed off in accordance with established safety procedures.
7. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
8. Spills should be decontaminated promptly with suitable disinfectant.
9. Take out the Cards from the pouch just before performing the test to avoid denaturation of antisera due to atmospheric exposure.

Optimal test performance requires strict adherence to the test procedure described in the insert.

PRECAUTIONS

1. Do not open the foil pouch to remove the product until it attains **room** temperature and you are ready to perform the test.
2. Do not freeze the product.
3. Interpret the result at the end of 20 minutes only.
4. Take out the Cards from the pouch just before performing the test to avoid denaturation of antisera due to atmospheric exposure.

Optimal test performance requires strict adherence to the test procedure described in the insert.

SAMPLE / SPECIMEN COLLECTION & STORAGE

a) HEPACARD should be performed on human serum or plasma only immediately after collection.

b) If not tested immediately, specimen should be refrigerated at 2-8°C upto 3 days following collection.

c) If testing within 3 days is not possible, specimen should be stored frozen at -20°C.

d) Specimen containing visible precipitates or cloudy specimens may give inconsistent test results. Such specimens should be clarified prior to testing by high speed centrifugation i.e. 10,000 rpm for 15 minutes before testing.

e) Haemolysed specimen or specimen with microbial contamination should be discarded and fresh aliquot should be collected.

TEST PROCEDURE

1. Bring the required number of **HEPACARD** foil pouches and specimen to room temperature prior to testing.
2. Take out **HEPACARD** device from the foil pouch.
3. Label the test card with patient's name or identification number.
4. Add 2 drops (70 µl) of human serum/plasma specimen into the sample well using the dropper provided (use separate dropper/microtip for each specimen).
5. Allow reaction to occur during the next 20 minutes.
6. Read results at 20 minutes.
7. Discard the HEPACARD immediately after reading result at 20 minutes, considering it to be potentially infectious.

INTERPRETATION OF RESULT

REACTIVE :
As shown in Fig.1, appearance of pink coloured line, one each in test region "T" and control region "C" indicates that the sample is REACTIVE for HBsAg. A difference of intensity in colour may occur between the Test line & Control line depending on the concentration of the HBsAg in the serum but this does not affect interpretation of the result. Faint test line also should be considered HBsAg reactive.

Fig. 1

Depending on the concentration of HBsAg, positive results may be observed within 60 seconds. However, to detect concentration around 0.5 ng to 1ng/ml and to confirm a negative result, the test result should be read only at 20 minutes. If the conc. of HBsAg in the sample is very high, only test line may be observed. This is due to Hook's effect. Such samples should be diluted 1:10 or 1:20 in normal saline & again re-run the test. Diluted sample should show both control & test line. In case, if control line does not appear or is faint dilute the sample further.

NON-REACTIVE :
As shown in Fig.2 appearance of one distinct pink line in the control region "C" only, indicates that the sample is "NON REACTIVE" for HBsAg.

Fig. 2

INVALID :
When neither control line nor test line appears on the membrane as shown in Fig.3, the test should be treated as invalid which may be because of following reasons:

Fig. 3

a) Improper storage at temperature other than the recommended temperature.

b) Wrong procedure.

c) Long atmospheric exposure of the test device after opening the pouch.

The test should be repeated using a new HEPACARD and test sample.

LIMITATIONS OF THE PROCEDURE

1. The HEPACARD is for *in vitro* diagnostic use only.
2. The test should be used for the detection of HBsAg in serum or plasma only and not in other body fluids.
3. **This is only a Screening test.** All reactive samples should be confirmed by confirmatory test. Therefore for a definitive diagnosis, the patient's clinical history, symptomatology as well as serologica data, should be considered. The results should be reported only after complying with above procedure.
4. Additional follow up testing using available clinical methods (along with repeat HEPACARD test) is required, if HEPACARD test is non-reactive with persisting clinical symptoms.
5. False positive results can be obtained due to the presence of other antigens or elevated levels of RF factor. This occurs in less than 1% of the samples tested.

PERFORMANCE CHARACTERISTICS

The performance of HEPACARD has been evaluated in house with fresh as well as frozen samples from low risk as well as high risk groups by using a panel containing 1400 nos. of known serum/ plasma samples including cross reacting samples. The results of all the samples with a defined HBsAg status were fully comparable with those of HEPACARD. The results of the in-house study done are as follows:

No. of Samples	Status	HEPACARD	HEPACARD
		+ ve	- ve
125	ELISA +ve	125	-
1275	ELISA -ve	8	1267

Sensitivity : 100% **Specificity : 98.75%**

Precision Within-run and between-run precisions have been determined by testing 10 replicates of seven HBsAg positive samples : 4 weak, 2 moderate positive, 1 strong positive and 2 HBsAg negative. The C.V.(%) of negative, weak, moderate positive and strong positive samples were within 10% of the time.

ANALYTICAL SENSITIVITY :

a) HEPACARD can detect Hepatitis B Surface Antigen in serum or plasma at a concentration of as low as 0.5 ng/ml at 20 minutes. It shows overall agreement of 99.8% with EIA techniques for sample having conc. 0.5 ng/ml or more.

b) All the eleven HBsAg subtypes can be detected positive with HEPACARD.

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an *in vitro* diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

BIBLIOGRAPHY

1. Blumberg, B.S., (1964) Bull. N.Y. Acab Med., 40:377
2. Blumberg B.S. etal. (1965) J.A.M.A. 191:541.
3. Caldwell C.W. etal., (1977) Clin. Chem. Acta. 31:305
4. Peterson, D.L. etal., (1982) J. Biol. Chem., 257(17): 10414.
5. Robin, E (1979) Fed. Proc. 33 (13) 2665.

WARNING : The "see Through Device" of HEPACARD has been developed as a result of intensive research. It's DESIGN IS REGISTERED and the WORLD PATENT INCLUDING INDIA has been applied for. Anyone copying the device design will render oneself liable for legal action.

In vitro diagnostic reagent, not for medicinal use

Manufactured & Marketed By:
DIAGNOSTIC ENTERPRISES
Plot No.: 26, Indl. Estate, Sector-1, Parwanoo - 173 220, (H.P.)
Phone: 01792-232253 E-mail: de@diagnosticenterprises.com

CARBOGEN®

RAPID PLASMA REAGIN (RPR) CARD TEST / CARBON ANTIGEN FOR SYPHILIS TESTING

SUMMARY

Syphilis is a sexually transmitted (venereal) disease caused by the spirochete *Treponema pallidum*. After infection the host forms *Treponemal antibodies to Treponema pallidum*, in addition, the host also forms Non Treponemal antilipoidal antibodies in response to the lipoidal material released from the damaged host cell. These antibodies are traditionally referred to as 'Reagins.'

The Rapid Plasma Reagin (RPR) / Carbon Antigen test is a macroscopic nonTreponemal flocculation test for the detection and quantitation of antilipoidal antibodies. Non-Treponemal tests like CARBOGEN® are of great value when used for screening and follow up of therapy.

REAGENTS

1. CARBOGEN® reagent: A particulate carbon suspension coated with lipid complexes.
2. Positive control, reactive with the CARBOGEN® reagent.
3. Negative control, non reactive with the CARBOGEN® reagent.

CARBOGEN® detects antilipoidal antibodies in serum or plasma. As against the conventional V.D.R.L. reagents, test samples do not require heat inactivation.

Each batch of reagent undergoes rigorous quality control at various stages of manufacture for its specificity, sensitivity and performance.

REAGENT STORAGE AND STABILITY

Store the reagent at 2-8°C. DO NOT FREEZE. Once opened the shelf life of the reagent vial is as described on the reagent vial label provided it is not contaminated. Do not use reagents after the expiry date. Avoid exposure to elevated temperatures and air, as the reagent is highly sensitive to denaturation and drying.

PRESENTATION

REF		10514005	10514050	10514100	10514250
RPR Carbon Ag/Tests		5.0 ml	50 tests	100 tests	250 tests
Control	+	-	0.4 ml	0.4 ml.	0.4 ml.
Control	-	-	0.4 ml	0.4 ml.	0.4 ml.
Disposable slides with eight reaction circles		32	7	13	32
Disposable sample / control dispensing pipettes		-	50	100	250
Rubber Teat		-	1	2	2
Reagent dropper for dispensing carbon antigen		1	1	1	1
Mixing stick ladder		-	2	4	10
Package Insert		1	1	1	1

PRINCIPLE

During the testing procedure, the specimen, serum or plasma is mixed with the CARBOGEN® reagent and allowed to react for eight minutes. If antilipoidal antibodies are present in the specimen, they will react with the CARBOGEN® reagent forming visible black floccules. If antilipoidal antibodies are not present in the specimens, there will be no flocculation.

NOTE

1. In vitro diagnostic reagent for laboratory or professional use only. Not for medicinal use.
2. The reagents contain 0.1% Sodium azide as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
3. The reagents that are derived from human source have been tested for HBsAg and Anti-HIV antibodies and are found to be non-reactive. However handle the material as if infectious.
4. CARBOGEN® RPR / Carbon Antigen should be gently but thoroughly mixed before testing to disperse the carbon particles uniformly and improve test readability.
5. Performance of the reagent must be verified with positive and negative controls and it is recommended that controls be run with each test series.
6. Accessories provided with the kit only must be used for optimum results.
7. Do not use damaged or leaking reagents.

SAMPLE COLLECTION AND STORAGE

1. No special preparation of the patient is required prior to sample collection by approved techniques. Hemolysed or lipemic samples are not suitable for testing.
2. Fresh serum or plasma should be used for testing.
3. Samples not tested immediately may be stored at 2-8°C for upto 48 hours.
4. Hazy samples should be centrifuged. Use clear supernatant for testing.

MATERIAL PROVIDED WITH THE RPR KIT

1. Carbon Antigen.
2. Positive control, reactive with the reagent.
3. Negative control, non-reactive with the reagent.
4. Disposable slides with eight reaction circles.
5. Disposable sample / control dispensing pipettes.
6. Mixing sticks.
7. Rubber teats.
8. Reagent Dropper for dispensing the Carbon Antigen.

ADDITIONAL MATERIAL REQUIRED,

Stop watch, High intensity light source, Isotonic saline, Pipettes, Test tubes, Mechanical rotor at 180 r.p.m. circumscribing a circle 2 cm in diameter on a horizontal plane.

Note: For CARBOGEN® Carbon Antigen 5.0 ml: - Item Nos. 2-7 listed above under RPR kit, would be required additionally.

TEST PROCEDURE

Bring reagent and samples to room temperature before testing.

Thoroughly mix the CARBOGEN® reagent suspension by gentle agitation before testing.

Qualitative Method

1. Pipette one drop (50 µl) of the test specimen, positive and negative controls onto separate reaction circles of the disposable slide using a sample-dispensing pipette.
2. Add one drop of well-mixed CARBOGEN® reagent next to the test specimen, positive control and negative control by using the reagent dropper provided with the kit. Do not let the dropper tip touch the liquid on the slide.
3. Using a mixing stick mix the test specimen and the CARBOGEN® reagent thoroughly spreading uniformly over the entire reaction circle.
4. Immediately start a stopwatch. Rotate the slide gently and continuously either manually or on a mechanical rotor at 180 r.p.m.
5. Observe for flocculation macroscopically at 8 minutes.

Quantitative Method

1. Using isotonic saline prepare serial dilutions of the test sample positive in the qualitative method 1:2, 1:4, 1:8, 1:16,1:32, 1:64, 1:128 and so on.
2. Perform the qualitative test procedure using each dilution as test specimen.
3. The titre is reported as the reciprocal of the highest dilution, which shows a positive test result.

INTEPRETATION OF TEST RESULTS

Qualitative methods

- Large and Medium black floccules against white background : Reactive
- Small black floccules against white background : Weakly Reactive
- No floccules, even grey background : Non reactive

Flocculation is a positive test result and indicates the presence of antilipoidal antibodies in the test specimen.

No Flocculation is a negative test result and indicates the absence of antilipoidal antibodies in the test specimen.

Quantitative Method

The titer of antilipoidal antibodies is the highest dilution of the test sample giving a positive test result.

REMARKS

1. Quantitative procedure must be performed to determine the response to treatment and detect reinfection.
2. False positive reactions occur not infrequently and have been attributed to a variety of acute and chronic conditions.
3. In absence of supporting clinical, historical or epidemiological evidence, reactive results must be confirmed with more specific Treponemal tests.

4. It is strongly recommended that results of the test should be correlated with clinical findings to arrive at the final diagnosis.
5. Dispose all used and contaminated material as per Standard Biohazard Safety Guidelines.
6. The reagent dropper provided for dispensing the Carbon Antigen should be thoroughly cleaned with distilled water and air dried after use, to ensure that it does not contaminate the reagent during subsequent use.
7. Very slight roughness should be interpreted as a negative test result.
8 Non-treponemal tests such as RPR are known to suffer from a high degree of biological false positives in many conditions such as pregnancy,malaria and many other infectious diseases.
9. Non-treponemal tests such as RPR are known to have prozone/hook effect in samples that have a high titre of reagents leading to a false negative result .It is usually recommended to run the tests in two dilutions i.e. with neat sample and 1:8 diluted samples.

PERFORMANCE CHARACTERISITICS

The results of 100 serum samples obtained with CARBOGEN® were compared with those obtained using commercial reagent (A) with similar characteristics and another commercial reagent (B) (modified VDRL reagent) with another method.

Test Result	CARBOGEN®	A	B
+VE	46	46	46
-VE	54	54	54

The results of CARBOGEN® correlate 100% with both the commercial reagents used for evaluation.
Repeatability and reproducibility (inter-assay and inter-lot) were evaluated on a number of VDRL negative and VDRL positive samples. No variations were found in the outcome of different tests.

WARRANTY

This product is designed to perform as described on the label and the package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

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2. Kasaliya S.S and Lambert N.G. , Colour coded antigen test for Syphilis, Applied Microbiology, 1974, 28, pgs 317-318.
3. Clinical diagnosis and management by laboratory methods, 17th Edition, edited by John Bernand Henry, pgs 1139-1142.
4. McGrew B.E. et.al, Automation of a flocculation test, Am. J. Clin. Path, 1968, 50, pgs 52-59.
5. Data on file: Coral Clinical Systems.

8. False positive results are likely if the test is read more than one minute after mixing on the slide test.
10. Any deviation in test procedure could result in variable results.
11. Since techniques and standardization vary from lab to lab one tube difference in tube titres can be expected.
12. Use a separate disposable tip for each sample to prevent cross contamination.
13. After usage the antigen suspension should be immediately recapped and replaced at 2-8°C.
14. It is recommended that results of the tests should be correlated with clinical findings to arrive at the final diagnosis.
15. The performance of the reagents should be validated occasionally using the positive control provided. Good physiological saline may be used as a negative control.

PERFORMANCE CHARACTERISTICS

1. The positive control antisera should produce 1+ or greater agglutination at 1: 80 in the slide and tube test when tested with the TYDAL® antigen suspensions.
2. The negative control should show no agglutination with any of the TYDAL® antigen suspensions.
3. Generally accepted performance characteristic of this type of test is 70% specificity and sensitivity.
4. Reproducibility of TYDAL® antigen suspensions is 100% (+/- one double dilution).

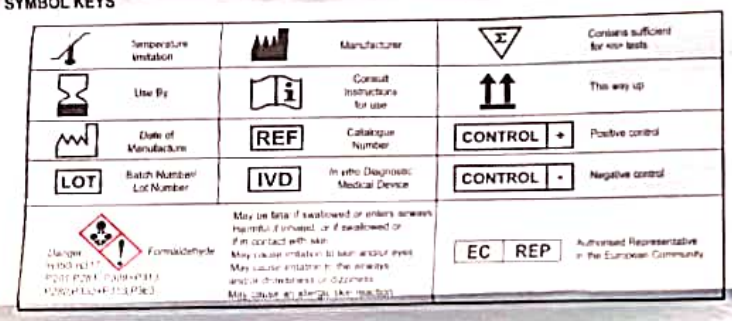
WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBILIOGRAPHY

(1). Cruickshank R., (1982). Medical Microbiology, 12th Edition, 403. (2).Felix A., (1942), Brt. Med. J., 11, 597-600. (3). Data on file: Tulip Diagnostics (P) Ltd.

SYMBOL KEYS



Manufactured by

TULIP DIAGNOSTICS (P) LTD.

Registered Office
GITANJALI, TULIP BLOCK, DR. ANTONIO DO REGO BARR, ALTO SANTACRUZ, BAMBOLIM COMPLEX P.O., GOA-403 202, INDIA. Website: www.tulipgroup.com

Manufacturing Unit
PLOT NO. UTILITY VIII, PHASE III B, VERNA IND. ESTATE, VERNA, GOA - 403 722, INDIA.

EC REP

CMC Medical Devices & Drugs S.L., C/ Horacio Lengo No. 18, CP 29006, Malaga, Spain

0529-8/0421/VER 05

CE

TYDAL®

WIDAL ANTIGEN SET / ANTIGENS FOR SLIDE AND TUBE TESTS

INTENDED USE

TYDAL® is a Widal slide and tube agglutination test that detects the presence of the serum agglutinins (O, H) in the patient's serum, with typhoid and paratyphoid fever.

SUMMARY

Enteric fever occurs when pathogenic microorganisms like *S. typhi*, *S. paratyphi* A, *S. paratyphi* B, *S. paratyphi* C infect the human body. During the course of disease, the body responds to this antigenic stimulus by producing antibodies whose titre rises slowly in early stages, to a maxima and then slowly falls till it is undetectable. Antibodies to *Salmonella* organisms may be detected in the patient serum from the second week after onset of infection. Information regarding the titres and whether or not they are rising or falling can be obtained by performing serological tests using TYDAL® antigen suspensions. Usually tube titres of 1:80 and above are taken as diagnostically significant, however for endemic areas higher cut-offs may need to be established.

REAGENT

TYDAL® contains ready to use concentrated, smooth antigen suspensions of the bacilli; *S. typhi* 'O', *S. typhi* 'H', *S. paratyphi* 'AO', *S. paratyphi* 'BO', *S. paratyphi* 'AH', *S. paratyphi* 'BH', *S. paratyphi* 'CH', *S. paratyphi* 'CO' and / or polyspecific positive control reactive with these antigens.
Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity and performance.

REAGENT STORAGE AND STABILITY

1. Store the reagents at 2-8°C. DO NOT FREEZE. Keep the reagents away from direct sunlight.
2. The shelf life of reagents is as per the expiry date mentioned on the reagent vial labels. Do not use beyond expiry date.
3. Once opened the shelf life of the reagent vial is as described on the reagent vial label provided it is not contaminated.

PRESENTATION

Σ		4x5 ml	8x5 ml*	2x5 ml	2x5 ml	2x5 ml	2x2x 5 ml	2x2x 5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
REF		105200045	105200085	105210025	105200025	105220025	105210225	105220225	105220005	105230005	105240005	105250005	105380005	105360005	105370005	105240005
Antigens		O, H, AH, BH	O, H, AH, BH, CH, AO BO, CO	O, H	O, H	O, H	O, H	O, H	O	H	AO	BO	CO	AH	BH	CH
Control	+	0.4 ml	2.0 ml	0.4ml	0.4ml	0.4ml	0.4ml	0.4ml								
Control	-		2.0 ml		0.4ml	0.4ml		0.4ml								
MIXING STICKS LADDER		4	6		4		4	4								
DISPENSER PP TUBES		50	50		50		50	50								
RUBBER TEAT		1	1		1		1	1								
SLIDE		1	1		1		1	1								
PACKAGE INSERT		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

* 8 x 5 ml pack is marketed as TYDAL® PLUS.

ADDITIONAL MATERIAL REQUIRED

Slide test method: Stop watch, Variable Micropipettes

Quantitative method: Timer, Kahn tubes / test tubes, Pipettes (0.1ml, 1ml), Physiological saline, Incubator (37°C), Test tube rack

PRINCIPLE

When the coloured, smooth, attenuated TYDAL® antigen suspensions are mixed / incubated with patient serum, anti-*Salmonella* antibodies present in the patient serum react with the antigen suspensions to give agglutination. Agglutination is a positive test result, indicating presence of anti-*Salmonella* antibodies in the patient serum. No agglutination is a negative test result indicating absence of anti-*Salmonella* antibodies.

NOTE

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. The *S. typhi* 'O', *S. paratyphi* 'CO' reagents contain 0.5% Phenol. *S. typhi* 'H', *S. paratyphi* 'AH', *S. paratyphi* 'CH' reagents contain 0.1% Formaldehyde and *S. paratyphi* 'AO', *S. paratyphi* 'BO' reagents contain 0.1% Ethanol along with 0.1% Sodium azide as preservatives. Avoid contact with skin and mucosa. Do not breathe vapour. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Sodium azide may react with lead and copper in plumbing and form highly explosive metal oxides, on disposal flush with large quantities of water.
3. The reagent can be damaged due to microbial contamination or on exposure to extreme temperatures. It is recommended that the performance of the reagent be verified with the positive and negative controls. Positive control provided with the kit only for TYDAL® 4 x 5 ml set (REF.: 105200045), 2 x 5 ml set (REF.: 105210025 & REF.105200025), 2 x 2 x 5 ml set (REF.: 105210225) and TYDAL® PLUS 8 x 5 ml set (REF.: 105200085). Negative Control provided with the kit only for 8 x 5 ml (REF.: 105200085), 2 x 5 ml (REF.105200025) and 2 x 2 x 5 ml (REF.105200225).
4. Shake the reagent vials well before use to disperse the antigen suspension uniformly and improve test readability.
5. Only clean and dry slides / tubes must be used. Clean the slide / tube with distilled water and dry.
6. It is necessary to use the calibrated dropper provided in the reagent vial to dispense a reagent drop.
7. TYDAL® antigen suspensions are not from human sources hence contamination due to HBsAg and HIV is practically excluded.
8. Accessories provided with the kit only must be used for optimum results. (Applicable only for TYDAL® 2 x 2 x 5 ml set (REF.: 105210225, 105200225), 2 x 5 ml set (REF.: 105200025), 4 x 5 ml set REF.: 105200045) and TYDAL® PLUS 8 x 5 ml set (REF.: 105200085).
9. Do not use damaged or leaking reagents.

SAMPLE COLLECTION AND STORAGE

1. No special preparation of the patient is required prior to sample collection by approved techniques. Do not use haemolysed and turbid samples.
2. Clean and dry glassware free from detergents must be used for sample collection.
3. Do not heat inactivate the serum.
4. Though freshly collected serum is preferable, store samples at 2-8°C in case of delay in testing, for upto 72 hours.

TEST PROCEDURE

Bring reagents and samples to room temperature before testing.
Shake and mix antigens well before dispensing.

Slide Screen Method

1. Place one drop of positive control onto a reaction circle of the slide.
2. Place 50 µl of physiological saline onto the next reaction circle of the slide.
3. Place one drop of patient's serum to be tested onto each of the required number of reaction circles.
4. Add one drop of appropriate TYDAL® antigen suspension to the reaction circles containing Positive control & physiological saline.
5. Add one drop of appropriate TYDAL® antigen suspensions to the reaction circles containing the patient's serum.
6. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
7. Rock the slide gently back and forth, and observe for agglutination macroscopically at one minute.

Slide Semi-Quantitative Method

1. Using a pipette place 80 µl, 40 µl, 20 µl, 10 µl, and 5 µl of patient serum to be tested on 5 different reaction circles on the slide. The corresponding titres obtained will be 1:20, 1:40, 1:80, 1:160, & 1:320 respectively.
2. Follow step No. 5-7 of slide screen method.

Note: This method is recommended for obtaining quick approximate titres only.

Quantitative Method

Tube-test Procedure

1. Take appropriate number of sets (as required, one set for each antigen suspension) of 8 Kahn tubes / test tubes and label them 1 to 8.
2. Pipette into tube No. 1 of all sets 1.9 ml of physiological saline.
3. To each of the remaining tubes (2 to 8) add 1 ml of physiological saline.
4. To tube No. 1 of all sets add 0.1 ml of serum sample to be tested and mix well.
5. Transfer 1 ml of the diluted serum sample from tube No. 1 to tube No. 2 and mix well.
6. Transfer 1 ml of the diluted serum sample from tube No. 2 to tube No. 3 and mix well. Continue this serial dilution till tube No. 7 in each set.
7. Discard 1.0 ml of the diluted serum from tube No.7 of each set.
8. Now the dilutions of the serum sample achieved from tube No. 1 to 7 respectively in each set is as follows: 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280. Tube No. 8 in all the sets, serves as a saline control.
9. To all the tubes (1 to 8) of each set add one drop of the respective well-mixed TYDAL® antigen suspensions from the reagent vials and mix well.
10. Cover and incubate at 37°C overnight (approximately 18 hours).
11. Dislodge the sedimented button gently and observe for agglutination.

INTERPRETATION OF RESULTS

Slide Screen Method

Agglutination is a positive test result and indicates presence of the corresponding antibody in the patient's serum. No agglutination is a negative test result and indicates absence of the corresponding antibody in the patient serum.

Slide Semi-Quantitative Method

Agglutination is a positive test result. The titre of the patient serum corresponds to the visible agglutination in the test circle with the least amount of serum sample.

Quantitative Method

The titre of the patient serum using TYDAL® antigen suspensions is the highest dilution of the serum sample that gives a visible agglutination.

REMARKS

1. Positive results obtained in the slide test should be confirmed with the tube test to establish whether the titres are diagnostically significant or not.
2. TAB vaccinated patients may show a high titre of antibodies to each of the antigens. Similarly, an amnestic response to other vaccines and unrelated fevers in case of patients who have had prior infection or immunization may give a false result.
3. Agglutinins usually appear by the end of the first week of infection, blood sample taken earlier may give a negative result.
4. A rising titre is more significant than a single high titre. It is therefore necessary to evaluate two or more serum samples taken at 4 - 6 days intervals after the onset of the disease.
5. 'O' being a somatic antigen brings about a coarse, compact, granular agglutination whereas 'H' being a flagellar antigen brings about larger, loose, flocculant agglutination.
6. While the 'O' antigens is species specific, the 'H' antigen is specific to the serotype.
7. Serological findings are not intended as a substitute for culture. An appropriate attempt should be made to recover and identify the etiologic organisms through various culture and biochemical tests.
8. Generally antibody titres of 1:80 or more are considered clinically and diagnostically significant. However the significant titre may vary from population to population and needs to be established for each area.