CAPTIA™ NMT Syphilis IgM

REF 2329360 CAPTIA™ NMT Syphilis IgM 96

Pour d'autres langues	Para outras linguas
Für andere Sprachen	Για τις άλλες λώσσες
Para otras lenguas	För andra språk
Per le altre lingue	For andre språk
Dla innych języków	For andre sprog

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INTENDED USE

This kit is intended for the detection of IgM specific antibodies to *Treponema pallidum (T. pallidum)* in human serum or plasma and is used in the differential diagnosis of syphilis infections.

SUMMARY AND PRINCIPLE

In infections with the spirochaete *T.pallidum* the primary response to the organism is the production of IgM class antibodies. This is followed by the appearance of IgG class antibodies which are detectable throughout all stages of the disease. Specific IgM antibodies tend to decline and eventually disappear – their presence is therefore a useful indicator of whether an infection is recent.

In conjunction with screening tests such as the CAPTIA™ Syphilis TA EIA (Ref: 2322170/2322171) the CAPTIA™ NMT Syphilis IgM can be used to aid in the diagnosis and investigation of cases of syphilis.

Maternal syphilis can be transmitted to the unborn foetus. IgM class antibodies cannot pass through the placenta, therefore detection of *T. pallidum*-specific IgM antibodies in neonatal blood samples can aid in the diagnosis of congenital syphilis.

Microtitration plate wells are supplied coated with anti-human IgM antibodies. IgM specific antibodies to *T. pallidum* present in a sample of plasma or serum will bind when the sample is incubated in the coated well. After unbound material is washed away, peroxidase-conjugated treponemal antigens are added, and in turn attach to any specific human antibodies captured.

After another washing step, a substrate/chromogen mixture is added to the wells, and the presence of the enzyme label is revealed by a colour change in the chromogen.

The optical absorbance of each well is read at appropriate wavelengths, and compared to controls to determine the presence or absence of anti-*Treponema pallidum* IgM antibodies.

REAGENTS

REAGENT DESCRIPTION

R1: Plate	Polystyrene coated with anti-human IgM. 96 wells in 12 strips of 8. 1 plate per kit.
R2: Sample buffer	Buffer containing protein and surfactant. Red. 20 mL.
R3: Conjugate	11x concentrate. *T. pallidum* antigens, peroxidase conjugated. Colourless/Pale Blue. 1.2 mL.
R4: Conjugate buffer	Buffer containing surfactant and stabilisers. Blue. 12 mL.
R5: Wash	20x concentrate. Saline containing surfactant. Colourless. 125 mL.
R6: Positive control	Positive human serum. 0.5 mL. Orange.
R7: Negative control	Negative human serum. 0.5 mL. Yellow.
R8: Substrate	Urea peroxide and tetramethyl benzidine. Pink. 12 mL.
R9: Stop solution	0.5M H_2SO_4. Colourless. 12 mL.
Instructions for Use	
Bag for storing unused wells	

ADDITIONAL MATERIALS REQUIRED

- Properly calibrated and maintained pipetting devices capable of delivering volumes of 10, 50 and 100 µL (specimens and reagents) and approx 300 µL (wash fluids).
- Plate or strip reader to read at 450 nm, 550 nm and 620 nm.
- 37°C incubator.
- Plate shaker

INSTRUMENTS

Captia™ Syphilis NMT IgM may be automated for both liquid handling and result interpretation. A variety of systems have been used for this, please consult the manufacturers of both the kit and the automation system for advice on automation.

Equipment should be able to support the following tolerances:

Volume dispensed	+/- 10%
Incubation temperature	+/- 2°C
Incubation time	+/- 2 minutes

STORAGE AND STABILITY

- All reagents as supplied may be used up to their expiry date if stored at 2-8°C in their original containers.
- Store bottles upright.
- Do not freeze.
- Do not expose substrate to direct sunlight.
- Diluted Wash is stable for 4 weeks at 2-8°C.
- Coated strips removed from the original packaging and stored in the bag provided at 2-8°C may be used for up to 4 weeks after opening.
- Make up sufficient diluted conjugate only for the number of strips to be run. The diluted conjugate is stable for 12 hours.

SPECIMEN COLLECTION AND STORAGE

- Serum or plasma (collected into EDTA, sodium citrate or heparin) samples may be used.
- Specimens may be stored at 2-8°C for up to 7 days before testing.
- Specimens needing longer storage should be frozen at -20°C or lower and be well mixed after thawing.
- Samples that are haemolysed, icteric or lipaemic may be run in the assay but the sample indicator system may not be relied upon.
- Samples that have been heated to 56°C for viral inactivation may be used in the assay.

WARNING AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- For professional use only.
- This test is designed to be used by appropriately trained laboratory personnel in the clinical laboratory.
- All human materials used have been tested and found negative for indicators of HIV, Hepatitis B, and Hepatitis C infection. They should nevertheless be regarded as potentially infectious and treated and discarded according to applicable regulations for such materials.
- Blood samples may contain pathogenic organisms. Gloves should be worn throughout, and all discarded materials sterilised by appropriate methods.
- All equipment should be properly maintained and calibrated according to the manufacturers' instructions.
- Do not combine or interchange reagents from kits with different lot numbers.
- Ensure that bottle caps are returned to the correct bottles.
- Do not use the kit after its expiry date.

PROCEDURE

PROCEDURAL NOTES AND PRECAUTIONS

- Bring all reagents and specimens to room temperature prior to use.
- Washing must be thorough, with complete filling and emptying of the wells at each cycle.
- The Negative control must be tested three times with each lot of tests, and the Positive control twice.
- Immediately after use return to recommended storage conditions.
- Take as many strips of wells as are needed for the number of samples to be tested, plus 5 wells for the controls. Store unused strips in the re-sealable plastic bag provided, and return to storage at 2-8°C.

REAGENT PREPARATION

- Dilute wash (R5) 1 in 20 with distilled or deionised water prior to use, plus the volume necessary for filling tubing, priming pumps, etc.
- Dilute conjugate (R3) 1 + 10 in Conjugate buffer (R4) (50 µL + 500 µL per 10 wells).
- Only prepare sufficient conjugate for the number of samples and controls being run; 50 µL of diluted conjugate is added per well. Discard any excess diluted conjugate after 12 hours. For example, for 2 strips add 100 µL concentrated conjugate to 1 mL conjugate buffer. Only dilute the quantity necessary for the assays.

TEST PROCEDURE

1. Add 100 µL **sample buffer (R2)** to each well followed by 10 µL of sample or control (R6 or R7) into each well as appropriate. Ensure the contents of the wells are mixed after sample addition.
2. Incubate at 37°C for 30 minutes, covered to reduce evaporation.
3. Aspirate the well contents and wash x5 with working strength wash ensuring complete filling and emptying at each cycle. If required, finally tap the inverted plate sharply on a layer of absorbent paper to remove any residual fluid.
4. Add 50 µL of **diluted conjugate (R3 + R4)** to each well.
5. Incubate at 37°C for 60 minutes, covered to reduce evaporation.
6. Wash x5 as described in step 3.
7. Add 50 µL **substrate/chromogen (R8)** mixture to each well.
8. Incubate at 18–25°C for 30 minutes, protected from light.
9. Add 50 µL **Stop solution (R9)** to each well, and mix (blue colour changes to yellow).
10. Within 10 minutes, read and record the absorbance of each well at 450 nm, preferably with a reference reading at 600-630 nm to eliminate the effects of any optical imperfection in the wells.

Verification of Sample and Reagent Addition

Visually:

Sample buffer will change from red/pink to clear/yellow upon addition of sample. Quality control sera which have been diluted during their manufacture may not give this colour change. The colour change is not quantitative for the amount of sample added.

Automatic Reading:

Addition of the samples and controls is verified by reading at 550 nm after the addition sample buffer and 450 nm after the addition of sample. An increase in optical density when compared to the blank reading of the sample addition buffer will occur for wells that have had sample added.

Addition of conjugate is verified by reading at 550 nm, a well with conjugate added must have an absorbance greater than 0.080.

Addition of substrate is verified by reading at 550 nm, a well with substrate added must have an absorbance greater than 0.080.

RESULTS

ASSAY VALIDATION

Mean Absorbance of the Negative controls must be <0.100 and each negative must give an antibody index <0.7.

Mean Absorbance of the Positive controls must be >1.200 and each positive must give an antibody index >8.0.

If any of the controls give results lying outside of these limits, the test should be repeated.

CUT-OFF CALCULATION

The Cut-Off Point (COP) is calculated as the mean of the Negative controls (NC) + 0.100 absorbance units.

i.e. $\frac{(NC1 + NC2 + NC3)}{3} + 0.100$

Example: $\frac{0.030 + 0.025 + 0.035}{3} = 0.030$

∴ Cut-Off Point = 0.030 + 0.100 = 0.130

The antibody index (AI) is determined by dividing the absorbance of the control or test sample by the cut-off point (COP).

$$AI = \frac{\text{absorbance of control or test sample}}{\text{Cut-off point}}$$

INTERPRETATION OF RESULTS

Samples giving AI values less than 0.9 are **NEGATIVE** for Syphilis IgM antibody.
Samples giving AI values greater than 1.1 are **POSITIVE** for Syphilis IgM antibody.
Samples giving AI values between 0.9 and 1.1 inclusive ($0.9 \leq EQL \geq 1.1$) are **EQUIVOCAL**.
If an equivocal result is obtained, the samples must be retested in duplicate. If upon retest equivocal results are obtained a fresh specimen should be requested.
The diagnosis of an active syphilis infection should not be made on the sole basis of a positive syphilis IgM result but in conjunction with other tests and clinical evidence.

PERFORMANCE CHARACTERISTICS

Precision

Intra and inter assay precision was assessed at 3 levels using a negative sample and 2 positive samples representing an intermediate and strong IgM response. Intra-assay precision was calculated using 48 replicate measurements at the levels indicated. Inter-assay precision was calculated by running the samples in quadruplicate over 5 different assays.

	Intra-assay %CV	Intra-assay %CV
Negative	11.7	12.4
Intermediate Positive	2.9	5.0
Strong Positive	3.6	7.6

Specificity

Clinical specificity was assessed by testing 87 plasma and serum samples from blood donors. The samples were tested using the CAPTIA™ NMT Syphilis IgM assay and a competitor assay. The CAPTIA™ NMT Syphilis IgM assay scored all samples as negative thus giving a specificity of 100%.

Sensitivity

Several studies were conducted comparing the performance of this kit with other commercially available CE marked kits on samples submitted to reference laboratories for specialist syphilis serology.

The combined results were as follows:

	IgM Pos (Both kits)	IgM Pos (CAPTIA™ NMT Syphilis IgM only)	IgM (Other kit only)
Confirmed case Other syphilis serology positive	26	7	7
Other syphilis serology negative	0	0	2

Additional sensitivity studies were performed using a syphilis seroconversion panel (Serologicals Inc., USA); 100% agreement between the CAPTIA™ NMT Syphilis IgM kit and two other commercially available syphilis IgM kits was obtained.

REFERENCES

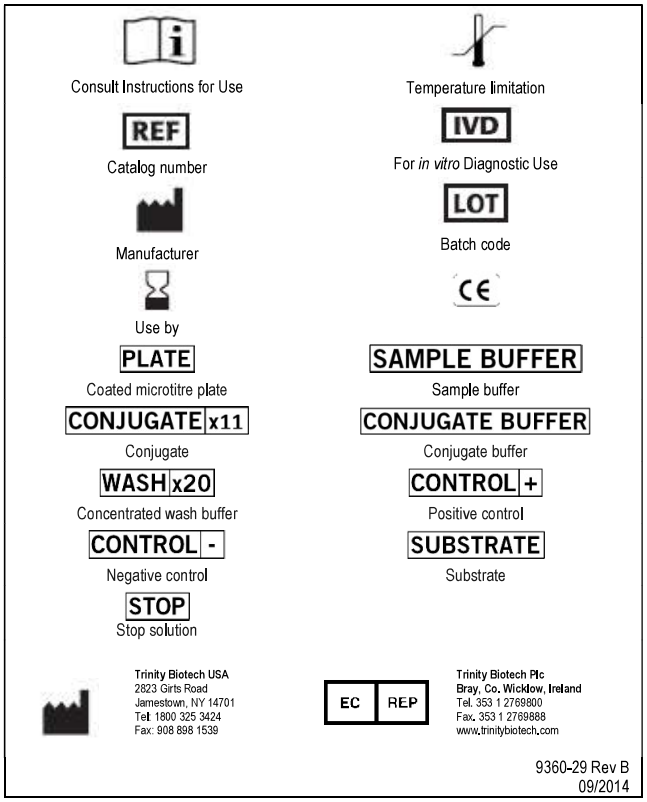
1. Larson S.A. et al Laboratory Diagnosis and interpretation of Tests for Syphilis. Clinical Microbiology Reviews 1995; 8: 1-21.
2. Pope V. et al A manual of tests for Syphilis.
3. Norris S.J. Polypeptides of Treponema pallidum: Progress toward understanding their structural, functional and immunological role. Microbiological Reviews, 1993;I 57:750-779.
4. Egglestone S.I. & Turner A.J.L. Serological Diagnosis of syphilis, Communicable Disease and Public Health 2000; 3: 158-162.

ORDERING INFORMATION

KIT

Kit Content	Item	Quantity
2329360	CAPTIA™ NMT Syphilis IgM	96 Tests

GUIDE TO SYMBOLS



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