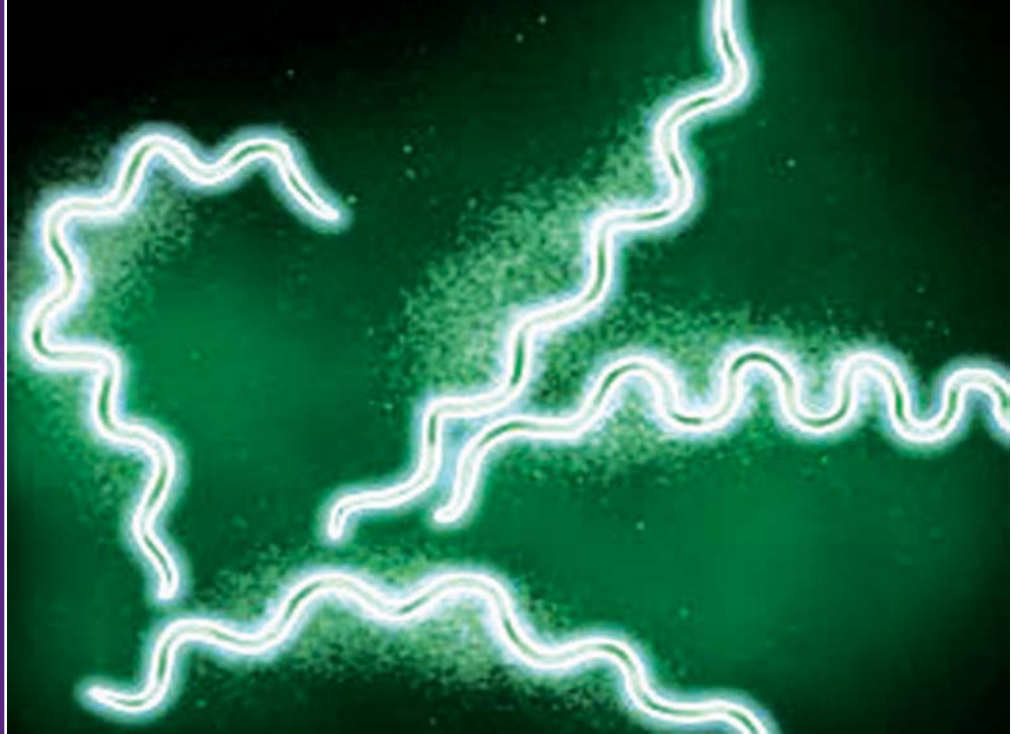


# syphilis screening tests



**RANDOX**



# **RANDOX** syphilis screening tests



Syphilis is a contagious systemic disease caused by the spirochete *Treponema pallidum*. The disease occurs in clinical stages, which are characterised by the production of reagins and anti-treponemal antibodies.

Many cases of syphilis remain undetected as symptoms mimic other more common conditions and, in the early stages, do not cause significant discomfort to the individual. Undiagnosed cases of syphilis provide greater risk of spreading the disease across the population.



## **Why screen for syphilis?**

In 1990 the World Health Organisation classed syphilis as one of the World's 50 most prevalent diseases, with 3.5 million new cases worldwide. 1999 statistics from WHO state that there were 12 million new cases worldwide.

The dramatic rise in the incidence of the disease, along with the ease with which it can be treated if diagnosed early, highlight the importance of carrying out widespread screening.



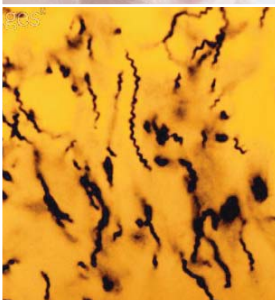
Syphilis screening is of great importance in:

- Controlling the spread of the disease
- Preventing the transmission of congenital syphilis
- Preventing irreversible tissue damage

Who should be screened for syphilis?

- Blood donors
- Pregnant women
- Patients attending sexually transmitted infection clinics.

Syphilis Factfile



**Clinical manifestations:** ulcerations of the uro-genital tract, mouth or rectum. There may be fever and general malaise, as well as hair loss and mild hepatitis in the later stages.

**Complications:** abortion, premature delivery, still birth, and neonatal or congenital syphilis, disorders of the musculo-skeletal, cardiovascular and nervous system (tertiary syphilis).

**Diagnosis:** Screening tests are simple and cheap.

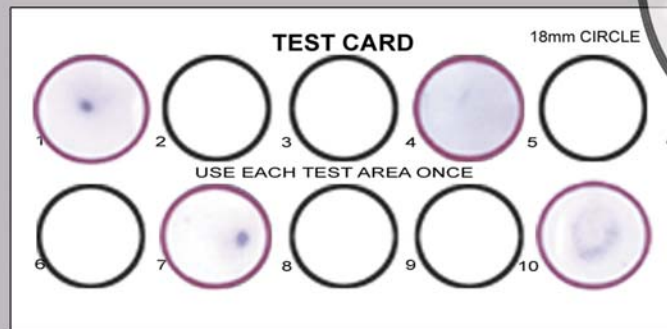
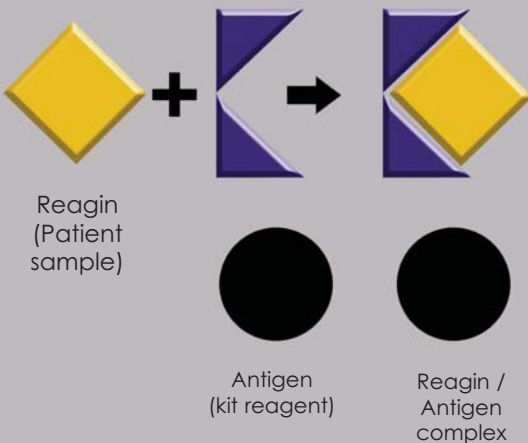
**Treatment:** Antibiotics are effective in the early stages.

# Reagin Tests (Non-Treponemal tests)

Reagins are antibodies produced in response to lipids set free when syphilis induced tissue damage occurs. As tissue damage occurs with other diseases, such as infectious mononucleosis, systemic lupus erythematosus, rheumatoid arthritis, infectious hepatitis and malaria, these tests are not specific for syphilis.

However, they are low cost, quick and simple to run and are therefore very useful as screening tests. The tests are of great value in monitoring the success of therapy, as reagin levels will decline over a period of 3-6 months when primary syphilis is treated. As reagins do not occur in the first few weeks of the infection, reagin tests lack sensitivity in the early stages of syphilis.

## RPR (Macroscopic) method

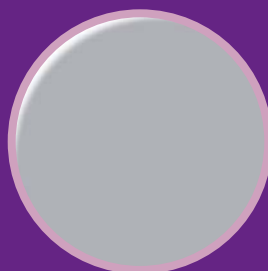


Positive sample appears as black aggregates, visible by eye



**negative**

Concentration of smooth carbon in the centre of the circle (no aggregates)



**negative**

Smooth even grey suspension



**weak positive**

Concentration of fine black aggregates at centre surrounded by a diffuse area



**positive**

Production of black aggregates without concentration at centre

The RPR antigen suspension is a modified version of the VDRL antigen, containing microparticulate carbon. This acts as a visualisation agent, enabling the flocculation that occurs in the presence of reagins to be seen macroscopically (by the naked eye).

### Features of the Randox RPR test

- Liquid reagents stable to expiry (2 years from manufacture).
- Positive and negative controls are included in our kits.
- The Randox RPR is a rapid 8 minute test.
- Various kit sizes are available to suit individual customer needs.
- The Randox RPR test has been calibrated against a CDC reference serum.

# Treponemal tests

TPHA is the main treponemal test used in the serodiagnosis of syphilis. It detects antibodies produced against *Treponema pallidum*, and is therefore highly specific.

Two types of antibodies are present during syphilis infection. **IgM** appears in the early stages of syphilis. IgM tests have good sensitivity in early syphilis, but this decreases in patients with longer term infections. Randox TPHA detects IgG.

**IgG** appears after the first few weeks and persists through all stages of the disease. TPHA and some ELISA tests detect IgG. IgG detection has greater sensitivity and specificity in the detection of syphilis. However, since these antibodies take a few weeks to appear, IgG tests lack sensitivity in the early stages of the disease.

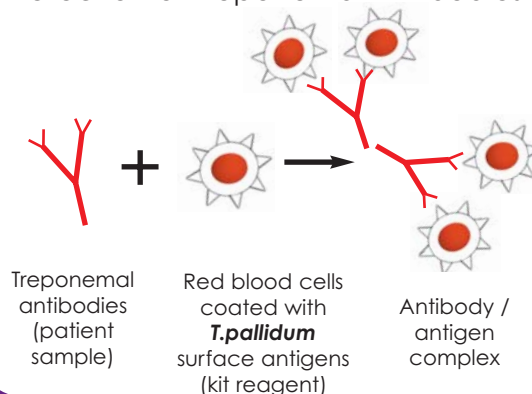
## TPHA Method

This is an indirect haemagglutination test for the detection and titration of the specific antibodies to *T. pallidum* that are produced following infection with the bacterium. Red blood cells (ovine or avian) that have been coated (sensitised) with cell-surface antigens of *T. pallidum* are added to the test serum. In a positive sample, antibodies to *T. pallidum* bind to the antigens on the erythrocytes. The cross-links that result cause the cells to agglutinate.

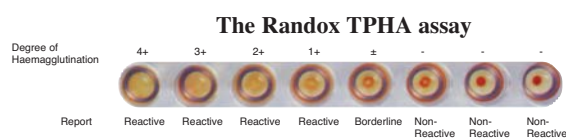
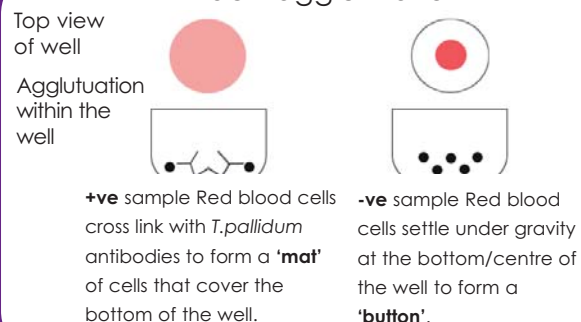
With a non-reactive serum, no agglutination occurs with the sensitised cells and they drop to the bottom of the well, forming a compact button.

TPHA is simple to perform, and is also suitable for use as a screening test. However, as it detects specific antibodies to *Treponema pallidum* it is very useful for confirming positive reactions with the non-treponemal tests. Although TPHA is not suited to monitoring treatment, when performed quantitatively, a four-fold increase in titre can indicate re-infection.

## Detection of Treponemal Antibodies

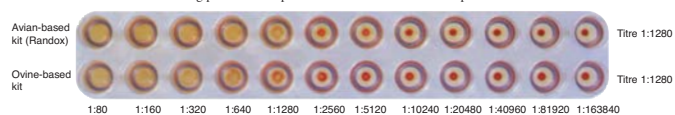


## Haemagglutination



## Randox TPHA assay compared with an ovine cell-based kit

With Randox avian cells the settling pattern is complete in a fraction of the time compared with the ovine cells.



## Randox TPHA assay - weak sample



## Randox TPHA assay - strong sample



Occasionally, very strong positive samples cause a distinctive haemagglutination pattern with folded edges in the first well (see below).



## Randox TPHA features

- The test utilises avian erythrocytes and therefore has a short reaction time of less than 60 minutes (reaction time can be up to 3.5 hours with ovine cell based TPHA kits)
- All reagents are in liquid ready-to-use format, and are stable to the expiry date
- Positive and negative controls are included in the kit
- The controls are supplied pre-diluted.
- Coloured diluent is provided to reduce the risk of errors when performing sample dilutions.
- The simple protocol reduces the number of dilution steps, reducing the risk of errors.
- Only 3 microtitre plate wells are required for the qualitative test.
- Reiter treponeme extracts present in sensitised test and unsensitised control cell suspensions to adsorb antibodies to non-pathogenic treponemes that could cause false positives.
- An absorption step is only necessary if there is a non-specific reaction between the sample and control cells.
- A bench reference card is available to aid with the interpretation of the settling patterns.
- The kit has a long shelf life, 18 months from manufacture.
- The Randox TPHA can be automated.

# Syphilis screening and confirmation

*Treponema pallidum* cannot be cultivated *in vitro* and therefore the standard way of detecting the disease is by performing serological tests. Syphilis testing includes screening techniques for the rapid throughput of numerous samples and then confirmation of the disease.

FTA-Abs is the current test used for confirmation of syphilis infection. This is an indirect fluorescence microscopy technique used to demonstrate antibody binding to the Nichol's strain of *T. pallidum*. This test is expensive, difficult to perform, time consuming and cannot be automated. This test is not practical for screening purposes.

## Screening tests

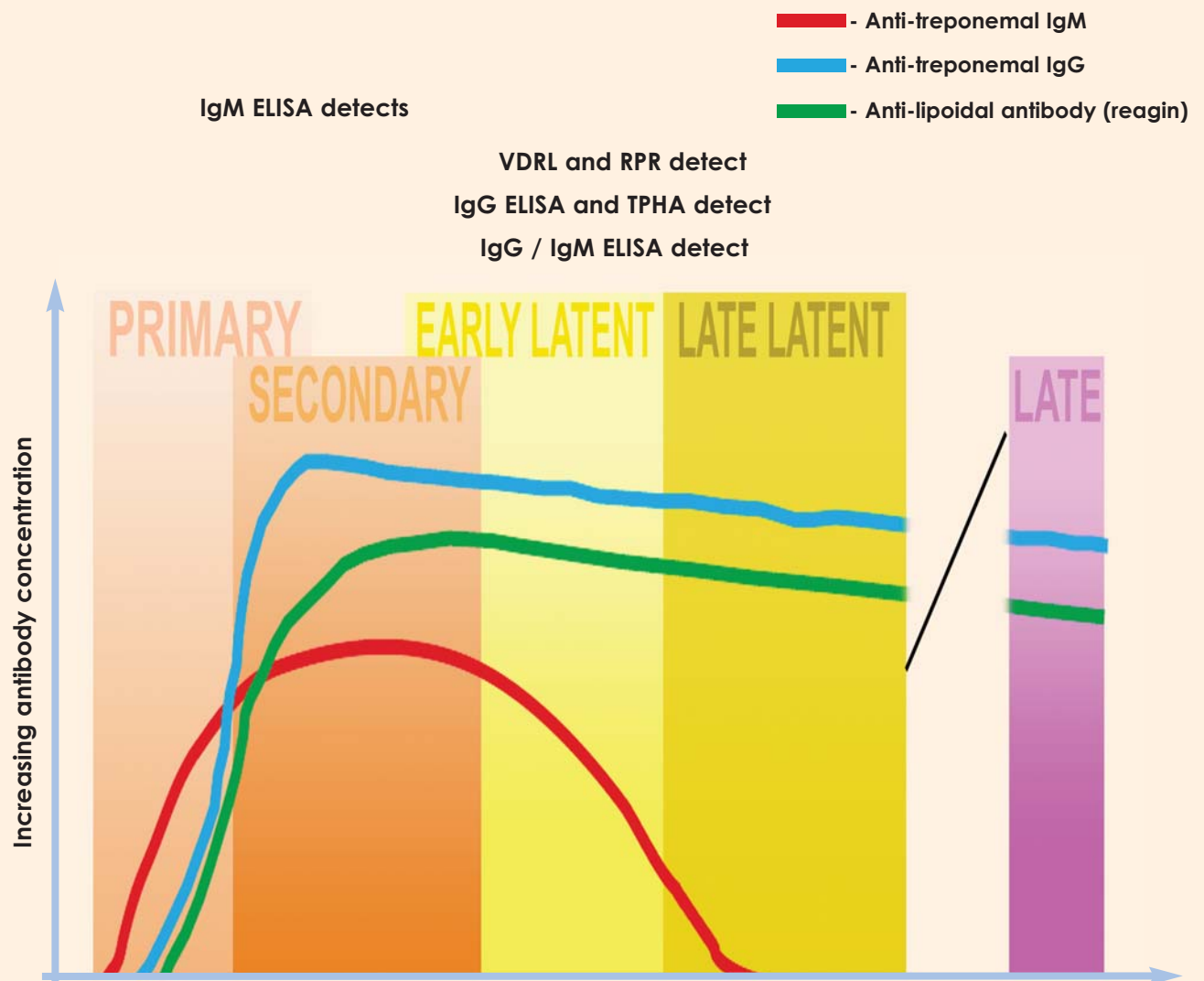
There are two classes of screening tests used for the detection of syphilis:

1. Non-treponemal tests using lipoidal antigens to detect syphilis reagins, such as the Venereal Disease Research Laboratory (VDRL) or the Rapid Plasma Reagin tests (RPR).
2. Specific treponemal tests detect anti-treponemal antibodies such as the *Treponema pallidum* Haemagglutination Assay (TPHA).

Studies have revealed that antibiotics are effective in the treatment of syphilis infections. The choice of serological tests for monitoring treatment is important.

When primary syphilis is treated reversal to seronegativity in reagin tests occurs in 3-6 months and therefore VDRL and RPR tests are excellent for monitoring the success of therapy. Anti-treponemal antibodies are much slower to decline than the reagins produced as a response to tissue damage.

**Appearance of antibody subtypes at various stages of syphilis, and the ability of various screening tests to detect different stages of infection.**



## External Evaluation of Randox TPHA kit

The Randox TPHA test was evaluated in a UK Public Health Laboratory and compared with five other commercially available kits.

- 486 reactive and non-reactive sera were used, including false positives.
- Randox test had an overall sensitivity of 97.6% and an overall specificity of 99.7%.
- The Randox assay scored the highest possible grading for sensitivity, specificity, clarity, positive and negative control sera, instruction booklet and differentiation between positives and negatives.

Category of sample	Number of samples	Specificity (%)
Antenatals	200	100
Known syphilis false positives	50	98
Potential false positives (includes RF positive sera, EBV sera and SLE sera)	50	100
Lyme disease sera	10	100
Leptospirosis sera	10	100

CAT.NO	PRODUCT DESCRIPTION	METHOD	SIZE
SY1478	RPR	CARD TEST	100T
SY1479	RPR	CARD TEST	500T
SY1480	TPHA	INDIRECT HAEMAGGLUTINATION	100T
SY1481	TPHA	INDIRECT HAEMAGGLUTINATION	200T
SY2215	TPHA SCREENING TEST	INDIRECT HAEMAGGLUTINATION	1000T

# RANDOX

International Headquarters  
Randox Laboratories Ltd., Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY  
Tel: +44 (0) 28 9442 2413, E-Mail: [marketing@randox.com](mailto:marketing@randox.com)

[www.randox.com](http://www.randox.com)

