LORNE LABORATORIES LTD.

GREAT BRITAIN

MICROTITRE PLATE HAEMAGGLUTINATION KIT

DIRECTIONS FOR USE

TPHA Microtitre Plate Kit: For The Qualitative Determination Of *Treponema pallidum*

SUMMARY

Syphilis is a venereal disease caused by the spirochaete micro-organism *Treponema pallidum.* This organism cannot be cultured on artificial media and so the diagnosis of syphilis depends on the correlation of clinical data with the specific antibody demonstrated by serological tests. There are two different techniques for the detection of syphilis. TPHA tests to detect antibodies to *Treponemal pallidum*, and non-treponemal serologic tests, to detect an antibody-like substance in infected people called Reagin.

INTENDED PURPOSE

The reagent is a latex test reagent intended to be used to qualitatively and semi-quantitatively determine the presence or absence of T.pallidum antibodies in the serum or plasma of patients when tested in accordance with the recommended techniques stated in this IFU.

PRINCIPLE

The TPHA (Trepenoma Pallidum Hemagglutination) is an indirect hemagglutination test for the qualitative and semi-quantitative detection of specific T.Pallidum antibodies in human serum. Stabilised avian erythrocytes sensitised with an antigenic T.Pallidum solution, agglutinates in the presence of T.Pallidum antibodies to give a characteristic pattern. No agglutination generally indicates absence of the antibodies (see **Limitations**).

KIT DESCRIPTION

Lorne TPHA Kit detects antibodies to T.pallidum. Test Cells are preserved avian erythrocytes coated with antigenic components of pathogenic T. pallidum (Nichol's strain). Any non-specific reactions are detected using the Control Cells; avian erythrocytes not coated with T.pallidum antigens. Non-specific reactions can also be absorbed out using Control Cells. Antibodies to non-pathogenic treponemes are absorbed by an extract of Reiter's treponemes in the cell suspension. The reagents do not contain or consist of CMR substances, or endocrine disrupting substances or that could result in sensitisation or an allergic reaction by the user.Reagents are supplied at optimal dilution for use with recommended techniques without need for dilution or addition. For lot reference number and expiry date see **Vial Labels**.

STORAGE

Do not freeze. Reagent vials should be stored at 2 - 8ºC on receipt. Prolonged storage at temperatures outside this range may result in accelerated loss of reagent reactivity. Store the vials in a vertical position. A horizontal position may cause cellular aggregates in reagents R1 and R2. If this happens mix the vials gently but thoroughly to disperse the aggregates.

SPECIMENS

Fresh serum or plasma. Stable up to 8 days at 2-8ºC or up to 3 months at –20ºC.
The samples with presence of fibrin should be centrifuged before testing. Do not use grossly hemolysed or lipaemic samples.

PRECAUTIONS

1. The kit is for *in-vitro* diagnostic use only.
2. Do not use kit past expiration date (see **Vial and Box Labels**).
3. Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
4. The reagents in this kit have been processed to reduce the bio-burden, but are not supplied sterile. Once a vial has been opened the contents should remain viable up until the expiry date.
5. No known tests can guarantee products derived from human or animal sources are free from infectious agents. Care must be taken in the use and disposal of each vial and its contents.

DISPOSAL OF KIT REAGENT AND DEALING WITH SPILLAGES

For information on disposal of kit reagent and decontamination of a spillage site see **Material Safety Data Sheets**, available on request.

CONTROLS AND ADVICE

1. It is recommended the TPHA Positive and Negative Controls be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
2. All the reagents must be allowed to reach 18-25ºC before use.
3. Mix the contents of each vial gently but thoroughly before use.
4. Avoid contamination of reagents or serum with saliva, as this will cause false positive results with specimens.
5. Do not interchange components between different kits.
6. Use of kit and interpretation of results must be carried out by properly trained and qualified personnel in accordance with the requirements of country where kit is in use and user must determine the suitability of the kit for use in other techniques.

KIT COMPONENTS SUPPLIED

1) R1: Test cells (Yellow cap, 1x7.5 mL): Stabilized avian erythrocytes sensitised with T.pallidum *(Nichols)* antigens, preservative, pH 7.2.
2) R2: Control cells (Green cap, 1x7.5 mL): Stabilized suspension of avian erythrocytes, preservative, pH, 7.2.
3) R3: TPHA Diluent (White cap, 2x10 mL): Phosphate buffered saline, pH 7.2, *T.pallidum* (Reiter) extract, preservative.
4) Control + (Red cap, 1x1 mL): Immune human serum prediluted 1:20, preservative.
5) Control – (Blue cap, 1x1 mL): Animal serum, preservative.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

a) Accurate pipettes.
b) U-well microtitre plates.

QUALITATIVE TECHNIQUE

1. Allow the reagents and sample to reach room temperature.
2. Shake both the Test and Control Cells vials gently but thoroughly immediately before use.
3. Dilute the sample 1:20 with Diluent (10 µL serum + 190 µL Diluent (R3)).
4. Pipette into adjacent wells of a microtitre plate:

	Test Well	**Control Well**
Sample 1:20 or Control*	25 µl	25 µl
Control Cells	--	75 µl
Test Cells	75 µl	--

*For each Positive Control or Negative Control or Patient Sample tested, 1 Test Well and 1 Control Well each is required.

5. Mix the microtitre plate thoroughly until a homogenous cells/sample is obtained.
6. Cover the microtitre plate and incubate at room temperature for 45-60 min. Keep the microtitre plate away from the vibrations, heat and direct sunlight.
7. Examine the agglutination patterns of the cells macroscopically.

SEMI-QUANITATIVE TECHNIQUE

1. Each specimen requires 8 wells of a microtitre plate, labelled A through to H.
2. Place 25 µl of Diluent into Wells B to H inclusive.
3. Transfer 25 µl of sample diluted 1:20, in **Qualitative Technique** above, to Wells A and B.
4. Take 25 µl of diluted sample from Well B and perform doubling dilutions of the sample from Wells B to H inclusive, discarding 25 µl of diluted sample from Well H.
5. Add 75 µl of Test Cells to Wells A to H inclusive.
6. Shake the microtitre plate gently to mix the contents thoroughly.

7. Cover the microtitre plate and incubate at room temperature for 45-60 min. Keep the microtitre plate away from the vibrations, heat and direct sunlight.
8. Examine the agglutination patterns of the cells macroscopically.

INTERPRETATION OF RESULTS

Read the results by comparing the agglutination patterns of the Test Cells with the Control Cells. Readings are scored and reported according to the following criteria:

Degree of hemagglutination	Reading	Result
Smooth mat of cells covering entire well bottom, sometimes with folded edges	4+	Reactive
Smooth mat of cells covering part of the well bottom	3+	Reactive
Smooth mat of cells surrounded by a red circle	2+	Reactive
Smooth mat of cells covering less area and surrounded by a smaller red circle	1+	Reactive
Button of cells having a small hole in centre	±	Borderline
Definite compact button of cells, sometimes with a very small hole in the centre.	-	Negative

INTERPRETATION OF RESULTS

1. The Negative Control should not show any agglutination pattern with both Test Cells and Control Cells.
2. The Positive Control should only show agglutination patterns with Test Cells.
3. Any agglutination pattern shown by Control Cells indicates the presence of non-specific antibodies and cannot be interpreted.
4. Samples with a borderline pattern should be retested and reported as negative if the same pattern is reproduced.
5. Reactive samples should be titred out as described in the semi-quantitative technique above. The serum titre is defined as the highest dilution showing reactive result.
6. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

LIMITATIONS

1. This kit cannot distinguish between syphilis and other pathogenic treponemal infections, e.g. Yaws and so clinical evidence should always be considered. It is recommended that all positive results are confirmed by an alternative method, such as FTA-ABS.
2. Lorne TPHA Syphilis Kit is highly specific but false positive results have been described with samples of patients with mononucleosis, leprosy, borreliosis, autoimmune diseases and drug addiction.
3. The TPHA test is not useful in determining the effectiveness of the therapy, since the antibody level remains (which would show a positive test result) sometime after the disease has been clinically cured.
4. False positive or false negative results may be seen due to:
   - Contamination of test materials
   - Improper storage of test materials or omission of reagent
   - Deviation from the recommended techniques

SPECIFIC PERFORMANCE CHARCTERISTICS

1. The kit has been characterised by procedures mentioned in the **Recommended Techniques**.
2. Prior to release, each lot of Lorne TPHA Syphilis Kit is tested by **Recommended Techniques** to ensure suitable reactivity.
3. Bilirubin (≤ 20 mg/dL), hemoglobin (≤ 10 g/L), lipids (≤ 10 g/L) and rheumatoid factors (≤ 300 IU/mL), do not interfere. Other substances may interfere[6].
4. **Analytical sensitivity**: 0.1 IU/mL, as tested against the 1st International Standard for human syphilitic plasma IgG and IgM NIBSC code 05/132.
5. **Prozone effect:** No prozone effect was detected up to titres ≥1/163840.
6. **Diagnostic sensitivity:** 100%
7. **Diagnostic specificity:** 100 %.

DISCLAIMER

1. User is responsible for performance of reagents by any method other than those mentioned in **Recommended Techniques**.
2. Any deviations should be validated prior to use using established laboratory procedures.

BIBLIOGRAPHY

1. David S.Jacobs et al. Laboratory Test Handbook, 3rd edition, Lexi-Comp Inc, 1994.

AVAILABLE KIT SIZES

Kit Size	Catalogue Number
100 Tests Per Kit	043100A

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