LORNE LABORATORIES LTD.

GREAT BRITAIN

SYPHILIS SEROLOGY KIT

DIRECTIONS FOR USE

VDRL Stabilised Reagent Kit: For Serodiagnosis Of Syphilis.

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, which detect antibodies to *Treponemal pallidum*, and non-treponemal serologic tests, which detect an antibody-like substance in infected people called Reagin.

INTENDED PURPOSE

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

PRINCIPLE

When used by the recommended techniques, the reagent will aggregate in the presence of reagin. No aggregation (no clumping) usually indicates the absence of reagin (see **Limitations**).

KIT DESCRIPTION

Lorne VDRL Stabilised Reagent Kit is a non-treponemal test for the serodiagnosis of syphilis. The kit consists of a stabilised VDRL antigen and a positive and negative control. The stabilised VDRL antigen is an ethanolic solution containing cholesterol (0.9%), bovine heart cardiolipin (0.03%) and lecithin (0.21%). 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. 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.

SPECIMENS

Fresh serum, plasma or cerebrospinal fluid. Stable 7 days at 2-8ºC or three months at -20ºC.
Samples with the presence of fibrin should be centrifuged before use. Do not use highly hemolized or lipemic samples. Specimens should be drawn with or without anticoagulant using an aseptic phlebotomy technique. If testing is delayed specimens can be stored at 2-8ºC for 7 days or for up to 3 months at or below –20ºC. Specimens must be free from bacterial contamination and haemolysis.

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.
6. Control +: H319 - Causes serious eye irritation. Follow the precautionary statement given in the SDS.

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 that the 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. Shake the reagents well before use to ensure homogeneity.
4. Do not interchange components between different kits.
5. The use of kit and the interpretation of results must be carried out by properly trained and qualified personnel in accordance with the requirements of the country where the kit is in use.
6. The user must determine the suitability of the kit for use in other techniques.

KIT COMPONENTS SUPPLIED

1) VDRL Antigen Stabilised (White cap, 5 mL); Solution containing Cardiolipin 0.3 g/L, Lecithin 2.1 g/L and Cholesterol 9 g/L in phosphate buffer 1.5 mmol/L, pH 7.0, preservative.
2) Control + (Red cap, 1 mL); Artificial serum with a Reagin titre ≥ 1/8.
3) Control – (Blue cap, 1 mL); Animal serum, preservative.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

a) Glass slides.
b) Adjustable pipette.
c) Mechanical rotator with adjustable speed of around 180 rpm.
d) Light microscope (10 x objective lens)

RECOMMENDED QUALITATIVE TECHNIQUE

1. Place in separate circles of the glass slide: 50 µl of undiluted patient serum, 50 µl of Positive Control and 50 µl of Negative Control.
2. Mix the VDRL stabilised antigen gently and add 20 µl of the VDRL reagent to each sample.
3. Mix the reagent and sample drops with a stirrer and spread over the entire area of each test circle.
4. Gently rotate the slide for four (4) minutes on a mechanical rotator set at 180 rpm.
5. After 4 minutes, examine each test circle for agglutination using a light microscope and record the results.

INTERPRETATION OF QUALITATIVE RESULTS

1) **Positive:** Visible agglutination using a light microscope constitutes a positive result and within accepted limitations of test procedure, indicates presence of Reagin associated with syphilis.
2) **Negative:** No visible agglutination using a light microscope constitutes a negative result and within the accepted limitations of the test procedure, indicates the absence of Reagin associated with syphilis.

RECOMMENDED SEMI-QUANTITATIVE TECHNIQUE

1. Make doubling dilutions of the specimen in 9 g/L saline up to 1/16 as follows:

Dilution	Serum	9 g/L Saline
1/2	100 µl undiluted serum	100 µl
1/4	100 µl undiluted serum	300 µl
1/8	100 µl undiluted serum	700 µl
1/16	100 µl undiluted serum	1500 µl

2. Test each serum specimen dilution in exactly the same way as for the **Qualitative Technique** above.

INTERPRETATION OF SEMI-QUANTITATIVE RESULTS

1. The titre is expressed as reciprocal of the highest dilution showing microscopic agglutination: e.g. if this occurs in dilution 1/8, the titre is 8.

INTERPRETATION OF RESULTS

1. **Reactive:** Medium to large clumps constitutes a positive test result and within the accepted limitations of the test procedure, indicates the presence of reagin.
2. **Weakly Reactive:** Finely dispersed particles with small clumps constitute a weak positive test result and within the accepted limitations of the test procedure indicates the presence of regain.
3. **Negative:** Finely dispersed particles with no clumps constitute a negative result and within the accepted limitations of the test procedure, indicates the absence of reagin.

STABILITY OF THE REACTIONS

Slide tests should be interpreted straight after the 4-minute rotation period to avoid the possibility that a negative result may be incorrectly interpreted as positive due to drying of the reagent.

LIMITATIONS

1. The VDRL test is non-specific for syphilis. All reactive samples should be retested withy treponemic methods such as TPHA and FTA-Abs to confirm the results.
2. A non reactive test result by itself does not exclude a diagnosis of syphilis.
3. False positive results have been reported in diseases such as infectious mononucleosis, viral pneumonia, toxoplasmosis, pregnancy and auto-immune disease.
4. Haemoglobin (≤ 10 g/L), bilirubin (≤ 20 mg/dL), lipaemia (≤ 10 g/L) and rheumatoid factors (≤ 300 IU/mL) do not interfere. Other substances may interfere[4].
5. False positive or false negative results may also occur due to:
   - Contamination of test materials
   - Improper storage of test materials or omission of reagents
   - Deviation from the recommended techniques

SPECIFIC PERFORMANCE CHARACTERISTICS

1. The kit has been characterised by the procedures mentioned in the **Recommended Techniques**.
2. Prior to release, each lot of Lorne VDRL Kit is tested by the **Recommended Techniques** to ensure suitable reactivity.
3. The reagent sensitivity is calibrated against the WHO 1st International Standard for human syphilitic plasma (NIBSC reference number 05/132).
4. Prozone effect: No prozone effect was detected up to titres of 1/128.
5. Diagnostic sensitivity: 100%.
6. Diagnostic specificity: 100%.

DISCLAIMER

1. The user is responsible for the performance of the kit by any method other than those mentioned by the manufacturer in the **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
250 Tests	046511A

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