### RETROSPECTIVE STUDIES: Suspected and Known Positive Syphilis Samples

A series of 412 total samples were processed from serum and blood center suppliers including 149 blank RPR and treponemal reactive serum samples and 26 serum samples that were requested to be Primary or Secondary Patients. Untreated or treated, but exhibiting a Syphilis-like lesion or rash were purchased from a serum supplier, and 130 frozen serum and plasma samples were obtained from a blood center. The samples were found to be RPR and treponemal reactive and having mixed titer. Another series of 97 samples being highly suspicious of having a syphilis infection were obtained from a serum supplier that were obtained from various laboratories around the U.S., and were submitted to the laboratories for testing. The samples were tested by RPR, TPPA, and MHA-TP as reference methods for comparison to Syphilis Health Check test.

The samples were further tested by an outside laboratory for TPPA and Syphilis Health Check results. Nineteen (19) samples were found to be Negative by TPPA and Syphilis Health Check reactive. Four samples were reactive by RPR. Ten samples were Suspected Positive for TPPA and no reactive samples were found by Syphilis Health Check. The samples were then found to be TPPA, Syphilis Health Check positive and reactive with the Syphilis Health Check test.

### PRECISION and REPRODUCIBILITY

Studies were performed to demonstrate the Intra-Assay, Inter-day, and Inter-Lot reproducibility of the Syphilis Health Check test kit. The within-run and between-day reproducibility of the Syphilis Health Check test were evaluated at three laboratories in order to assess the performance using a panel of 28-pooled samples. Each testing site conducted reproducibility studies using a supplied panel of samples ranging from non-reactive to reactive, i.e. one non-reactive, one high (1:256) reactive non-treponemal, one low (1:8) reactive, one moderate reactive, and two mid-high to high reactive in addition to the kit controls. For the between-day reproducibility evaluation, each site ran these panel member samples for at least five days, twice per day per operator. For the within-run reproducibility evaluation, each operator conducted the three levels per day for each panel member in one day.

The number of disease condition categories and reactive results obtained is listed in the following table. The two critical borderline samples near the cut-off yielded the following results.

### Potential Cross Reactors:

- Bilirubin (total): No effect was observed up to 40 mg/dL of total bilirubin;
- HbsAg: No effect was observed up to 1000 mIU/mL of HbsAg;
- HIV: 1% of the 2000 sera tested showed limited reactivity with serum titers up to 1:640;
- CMV: 1% of the 2000 sera tested showed limited reactivity with serum titers up to 1:640;
- Chlamydia: 1% of the 2000 sera tested showed limited reactivity with serum titers up to 1:640;
- HCV: 1% of the 2000 sera tested showed limited reactivity with serum titers up to 1:640;
- Hepatitis B virus: 1% of the 2000 sera tested showed limited reactivity with serum titers up to 1:640;
- Rs: 1% of the 2000 sera tested showed limited reactivity with serum titers up to 1:640;
- ALB: 1% of the 2000 sera tested showed limited reactivity with serum titers up to 1:640;
- AmHb: 1% of the 2000 sera tested showed limited reactivity with serum titers up to 1:640;
- Anti Hbs: 1% of the 2000 sera tested showed limited reactivity with serum titers up to 1:640;"
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