Hepatitis B surface antigen Ab ELISA Kit

Catalog Number KA0287
96 assays
Version: 32

Intended for research use only
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Introduction

Intended Use

The Hepatitis B surface antigen Ab ELISA Kit is an enzyme immunoassay kit for qualitative detection of Antibody to Hepatitis B surface antigen (Anti-HBs) in human serum or plasma (heparin, citrate or EDTA).

Background

Hepatitis B surface antigen (HBsAg) is the first antigen to appear following infection by hepatitis B virus. The development of antibodies against HBsAg (anti-HBs) occurs in 90% of patients infected with HBV late in convalescence approx. 3 to 4 months after the onset of the disease and is associated with resolution of the infection and protective immunity. The absence of anti-HBs is indicative of susceptibility to HBV infection, and can identify individuals who may benefit from vaccination. WHO reports that people with an anti-HBs titer of 10 mIU/mL can be assumed to be protected against HBV infection. The measurement of anti-HBs is useful for pre-immunization screening as well as to establish seroconversion after an infection or following active immunization with hepatitis B vaccines. Anti-HBs titers of < 100 mIU/mL will identify inadequate responders who require booster vaccination within one year. In addition, anti-HBs testing is useful to monitor the course of disease following acute HBV infection.

Principle of the Assay

The Hepatitis B surface antigen Ab ELISA Kit is a fast test for the qualitative detection of the presence of antibodies to HBsAg in serum or plasma (heparin, citrate or EDTA) specimen. The test utilizes HBsAg on the wells as peroxidase-conjugate.

Specimens which are non-reactive by the Hepatitis B surface antigen Ab ELISA Kit are considered negative for Anti-HBs. Specimens with positive reaction should be retested in duplicate.

The test has to be repeated in duplicate for specimens with absorbance value within the Retest Range (Cutoff Value ± 10 %).

The Hepatitis B surface antigen Ab ELISA Kit is a solid-phase enzyme immunoassay (ELISA= enzyme-linked immuno-sorbent assay) based on the "sandwich principle". The solid phase of the microtiter plate is made of polystyrene wells coated with HBsAg (subtype Ad and Ay), and the liquid phase of peroxidase conjugated HBsAg (subtype Ad and Ay).

When a serum or plasma specimen containing Anti-HBs is added to the HBsAg-coated wells together with the peroxidase conjugated HBsAg and incubated, (antigen)-(Anti-HBs)-(antigen • Peroxidase) complexes will form on the wells. After washing the microtiter plate to remove unbound material, a solution of TMB substrate is added to the wells and incubated. A color develops in proportion to the amount of Anti-HBs bound to HBsAg. The peroxidase-TMB reaction is stopped by addition of sulfuric acid. The optical density of developed color is read with a suitable photometer at 450 nm with a selected reference wavelength within 620 to 690 nm *1.
The above test principle is shown also in the following figure.

A. Specimen containing Anti-HBs:
1. Plate well (HBsAg) + specimen (Anti-HBs) + HBsAg • Peroxidase → HBsAg • Anti-HBs • (HBsAg • Peroxidase) sandwich complex
2. Sandwich complex + TMB substrate solution → Light blue to blue color
3. Add sulfuric acid to stop the color development → Read OD at 450 nm/620-690 nm *1

B. Specimen without Anti-HBs:
1. Plate well (HBsAg) + specimen (no Anti-HBs) + HBsAg • Peroxidase → HBsAg (on the well)
2. HBsAg (on the well) + TMB substrate solution → Colorless to light blue color
3. Add sulfuric acid to stop the color development → Read OD at 450 nm/620-690 nm *1

Note: *1 The reference wavelength of the photometer to be used can be 620 nm to 690 nm. However, the user should validate the photometer in combination with the Hepatitis B surface antigen Ab ELISA Kit before use.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg Plate</td>
<td>Microtiter plate coated with HBsAg.</td>
<td>1 plate</td>
</tr>
<tr>
<td>HBsAg • Peroxidase Solution</td>
<td>HBsAg • HRPO conjugate, diluted in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.</td>
<td>8 mL</td>
</tr>
<tr>
<td>Anti-HBs Positive Control</td>
<td>Inactivated human Anti-HBs positive serum diluted in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.</td>
<td>1.5 mL</td>
</tr>
<tr>
<td>HB Negative Control</td>
<td>Serum non-reactive for HBV markers in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.</td>
<td>2 mL</td>
</tr>
<tr>
<td>TMB Substrate Solution A</td>
<td>0.6 mg/mL of 3, 3’, 5, 5’-tetramethylbenzidine (TMB) in an organic base</td>
<td>12 mL</td>
</tr>
<tr>
<td>TMB Substrate Solution B</td>
<td>Citrate Acid Buffer containing 0.03% H₂O₂</td>
<td>12 mL</td>
</tr>
<tr>
<td>Conc. Washing Solution D (20x)</td>
<td>Concentrated Phosphate buffer with Tween-20</td>
<td>58 mL</td>
</tr>
<tr>
<td>Stop Solution 2</td>
<td>2 N H₂SO₄ (Sulfuric Acid)</td>
<td>12 mL</td>
</tr>
</tbody>
</table>

Accessories: (provided as needed)

<table>
<thead>
<tr>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesive slips</td>
</tr>
<tr>
<td>Absorbent pads</td>
</tr>
<tr>
<td>Black cover</td>
</tr>
</tbody>
</table>

Storage Instruction

- The kit must be stored at +2 to +8°C. Do not freeze.
- Strips of the plate should be used within 2 months after opening the original aluminum foil bag. The unused strips should be kept in the aluminum foil bag and taped tightly.
- Return reagents to +2 to +8°C immediately after use.
- Conc. Washing Solution D (20X) can be stored at room temperature to avoid crystallization, because the kits are stored at +2 to +8°C. If crystals have been precipitated before use, warm up the solution in 37°C water bath till the crystals dissolve.
Storage condition and Stability of the kit and components

<table>
<thead>
<tr>
<th>Kit/components</th>
<th>Storage temp.</th>
<th>State</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B surface antigen Ab ELISA Kit</td>
<td>2 - 8°C</td>
<td>Original</td>
<td>18 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once open</td>
<td>1 month</td>
</tr>
<tr>
<td>Anti-HBs Positive Control</td>
<td>2 - 8°C</td>
<td>Original</td>
<td>18 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once open</td>
<td>1 month</td>
</tr>
<tr>
<td>HB Negative Control</td>
<td>2 - 8°C</td>
<td>Original</td>
<td>18 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once open</td>
<td>1 month</td>
</tr>
<tr>
<td>HBsAg Plate</td>
<td>2 - 8°C</td>
<td>Original</td>
<td>24 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once open</td>
<td>2 months</td>
</tr>
<tr>
<td>HBsAg • HRPO Conjugate Solution</td>
<td>2 - 8°C</td>
<td>Original</td>
<td>18 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once open</td>
<td>1 month</td>
</tr>
<tr>
<td>Conc. Washing Solution D (20X)</td>
<td>Room temp.</td>
<td>Original</td>
<td>24 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once open</td>
<td>1 month</td>
</tr>
<tr>
<td>20X Diluted Washing Solution</td>
<td>Room temp.</td>
<td>Diluted</td>
<td>2 days</td>
</tr>
<tr>
<td></td>
<td>2 - 8°C</td>
<td>Diluted</td>
<td>1 week</td>
</tr>
<tr>
<td>TMB Substrate Solution A</td>
<td>2 - 8°C</td>
<td>Original</td>
<td>24 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once open</td>
<td>1 month</td>
</tr>
<tr>
<td>TMB Substrate Solution B</td>
<td>2 - 8°C</td>
<td>Original</td>
<td>24 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once open</td>
<td>1 month</td>
</tr>
<tr>
<td>Stop Solution 2</td>
<td>Room temp.</td>
<td>Original</td>
<td>24 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once open</td>
<td>1 month</td>
</tr>
</tbody>
</table>

Materials Required but Not Supplied

✓ 50 µL, 100 µL micropipettes and tips are needed.
✓ Waterbath or incubator with temperature control at 37°C.
✓ Plate washing equipment.
✓ ELISA microwell reader:
  Dual wavelength 450 nm with 620-690 nm as reference wavelength, bandwidth 10 nm*1.
✓ Fully automatic EIA micro-plate analyzer is optional. User should validate the automatic EIA microplate analyzer in combination with the kit.

*Note: *1 The reference wavelength of the photometer to be used can be 620 nm to 690 nm. However, the user should validate the photometer in combination with the Hepatitis B surface antigen Ab ELISA Kit before use.
Precautions for Use

✓ This reagent kit is for professional use only.
✓ This reagent kit is for research use only.
✓ Bring all kit reagents and samples to room temperature (+20 to +30°C) and mix carefully before use.
✓ Do not use reagent beyond its expiration date.
✓ Do not interchange reagents between different lots.
✓ Do not put the pipette in your mouth.
✓ Do not smoke or eat in areas where specimens or reagents are handled.
✓ The positive control, negative control, conjugate solution and specimens should be regarded as potential health hazards. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures may include the wearing of protective gloves and avoiding the generation of aerosols.
✓ Potential infectious specimens and nonacid containing spills or leakages should be wiped up thoroughly with 5% sodium hypochlorite or treated in accordance with the local procedures for potential bio-hazard control.
✓ Prior to disposing used specimens and kit reagents as general waste, it should be treated in accordance with the local procedures for potential bio-hazardous waste or treated as follows:
  Both liquid and solid waste should be autoclaved maintaining +121°C for at least 30 minutes.
  Solid waste can also be incinerated.
  Non-acidic liquid waste can be treated with sodium hypochlorite diluted to a final concentration of 1%.
  Acidic liquid wastes must be neutralized before treatment with sodium hypochlorite as mentioned above and should stand for 30 minutes to obtain effective disinfection.
✓ 2 N sulfuric acid is an irritant to skin, eyes, respiratory tract and mucous membranes. Avoid contact of the 2 N sulfuric acid with skin and mucous membranes. In case of contact, clean with large lots of water immediately. In case of inhalation, supply fresh air and seek medical advice in case of complaints.
✓ TMB substrate solution A contains organic solvent, which is flammable. TMB substrate solution A contains dimethyl sulfoxide, an irritant to skin and mucous membranes.
✓ Although all material of human source is tested non-reactive for Anti-HCV and Anti-HIV, and inactivated at +56°C for one hour, the reagent shall be handled as potential infectious material².
✓ Limitations and Interferences
  • This reagent kit is to be used for unpooled human serum or plasma only.
  • Non-repeatable false positive results may be obtained with any enzyme immunoassay kit, largely due to technical error either from the part of the operator or malfunction of apparatus used.
  • Repeatable false reactive results (≤ 2%) may occasionally be obtained.
  • An Anti-HBs negative result without other evidence does not preclude the possibility of previous infection with hepatitis B virus.
  • A (low) positive result in this kit is no proof of protection and such it should be not used to exclude
an infection by hepatitis B virus.

- Anti-HBs positive specimens may not always show linear serial dilution properties as in serial dilution of standard material.

- Potential Interfering Substances:
  The following results were obtained in respective experiments:
  1. No interferences with different anticoagulants such as lithium heparin, K-EDTA, sodium citrate have been observed.
  2. Heat-treated specimens (+60°C, 10 hours) exhibited diminished HBsAg titer.
  3. No cross reactivity was detected using specimens deriving from persons a) with other infections by HAV, EBV, CMV, HSV, VZV, Lyme Borreliosis, HCV, HIV, b) with other disease states such as chronic renal failure, hemodialysis, autoimmune hepatitis, liver cirrhosis, and c) presenting certain antibodies like HAMA, GAD, IA2, APS).
  4. Samples containing potential interfering substances [e.g. triglycerides (lipemia), hemoglobin (hemolysis), bilirubin (icterus), monoclonal immunoglobulin components, elevated levels of autoimmune antibodies (rheumatoid factor-RF, antinuclear antibodies-ANA, or antimitochondrial antibodies-ANA)] and samples from pregnant women did not interfere with the Hepatitis B surface antigen Ab ELISA Kit assay.

*Note: **2 Incomplete inactivation of hepatitis B virus after heat treatment at +60°C for 10 hours, J. Infect. Dis. 138:242-244.
Assay Protocol

Reagent Preparation

✓ Plate Washing Procedure
  • Preparation of washing solution:
    Dilute Conc. Washing Solution D (20X) with distilled or de-ionized water to 1:20 dilution. Do not use tap water.
  • Plate washing:
    1. For plate washer with overflow aspirating function: 6 cycles with at least 0.5 mL washing buffer per well per cycle.
    OR
    2. For plate washer without overflow aspirating function: 8 cycles with at least 0.35 mL washing buffer per well per cycle.
  • Blot dry by inverting the plate and tapping firmly onto absorbent paper. Too much residual wash buffer in the wells will cause false results.
    *Note: Improper washing will cause false results.*

Sample Preparation

✓ Specimen Collection and Preparation for Analysis
  • No special preparation of the person is required prior to blood collection. Blood should be collected by approved techniques.
  • Either serum or plasma specimens can be used with this test kit. Whole blood specimen should be separated as soon as possible in order to avoid hemolysis. Any particulates (e.g. fibrin clots, erythrocytes) contained in the specimen should be removed prior to use.
  • Specimens must be stored at +2 to +8°C and avoid heat-inactivation to minimize deterioration. For long-term storage, specimens should be frozen below -20°C. Storage in self-defrosting freezer is not recommended.
  • Frozen specimens must be thoroughly thawed and mixed homogenously before testing.
  • Avoid multiple freeze-thaw procedures.
    *Note:*
    1. The specimen must not contain any AZIDE compounds, which can inhibit the peroxidase activity of the conjugate.
    2. Incompletely coagulated serum samples and microbial-contaminated specimens should not be used.
Assay Procedure

1. Bring all reagents and specimens to room temperature (20 to 30°C) before assay. Adjust water bath or incubator to +37 ± 1°C.

2. Reserve one well for blank. Add 50 µL of each control or specimen to appropriate wells of reaction plate (3 Negative Controls and 2 Positive Controls).

   **NOTE:**
   1. *Use a clean pipette tip for each sampling to avoid cross-contamination.*
   2. *Each plate needs its own negative controls, positive controls and blank well.*
   3. Do not use any cut-off value established for other plates of the Hepatitis B surface antigen Ab ELISA Kit.

3. Add 50 µL of HBsAg Peroxidase solution to each well except the blank.

   **NOTE:** *Do not touch the well wall for preventing contamination.*

4. Gently tap the plate.

5. Remove the protective backing from the Adhesive Slip and press it onto the reaction plate, so that it is tightly sealed.

6. Incubate the reaction plate in a 37 ± 1°C water bath or incubator for 1 hour.

7. At the end of the incubation period, remove and discard the Adhesive Slip and wash plate in accordance with "PLATE WASHING PROCEDURES".

8. Select one of the following two methods for color development:
   - Mix equal volumes of TMB Substrate Solution A and B in a clean container immediately prior to use. Add 100 µL of the mixture solution to each well including the blank well.
   - Add 50 µL of TMB Substrate Solution A first, and then add 50 µL of TMB Substrate Solution B into each well including blank. Carefully mix well.

   **NOTE:** *TMB Substrate Solution A should be colorless to light blue, otherwise, it should be discarded. The mixture of TMB Substrate Solution A and B should be used within 30 minutes after mix. The mixture should be avoided from intense light.*

9. Cover the plate with a black cover and incubate at room temperature for 30 minutes.

10. Stop the reaction by adding 100 µL of 2 N H₂SO₄ to each well including the blank.

11. Determine the absorbance of Controls and test specimens within 30 minutes with a precision photometer at 450 / 620-690 nm (450 nm reading wavelength with 620-690 nm reference wavelength)*¹. Use the blank well to blank photometer.

   **Note:**
   *¹ The reference wavelength of the photometer to be used can be 620 nm to 690 nm. However, the user should validate the photometer in combination with the Hepatitis B surface antigen Ab ELISA Kit before use.
   1. *The color of the blank should be colorless to light yellowish; otherwise, the test results are invalid.*
   2. *Substrate blank: absorbance value must be less than 0.100.*
Flow chart of the test procedure

Add 50 µL controls (3 x NC, 2 x PC) and add 50 µL of each specimen into wells. Reserve one well for blank.

Add 50 µL of HBsAg • Peroxidase Solution into each reaction well, except one blank.

Incubate the plate at 37± 1°C for 1 hour.

Wash the plate.
(Choose one of the following two methods for color development)

Mix equal volumes of TMB Substrate Solution A and B.
Add 100 µL of the mixed solution to wells.

Add 50 µL of TMB Substrate Solution A to wells and then add 50 µL of TMB Substrate Solution B. Mix well, gently.

Incubate at RT for 30 minutes.

Add 100 µL of 2 N sulfuric acid into each well.

Determine absorbance using 450 nm as reading wavelength with 620-690 nm reference wavelength*1.

Note: *1 The reference wavelength of the photometer to be used can be 620 nm to 690 nm. However, the user should validate the photometer in combination with the Hepatitis B surface antigen Ab ELISA Kit before use.
Data Analysis

Calculation of Results

✓ Calculation of the NCx (Mean Absorbance of Negative Control).
Example:

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.015</td>
</tr>
<tr>
<td>2</td>
<td>0.016</td>
</tr>
<tr>
<td>3</td>
<td>0.014</td>
</tr>
</tbody>
</table>

\[ NCx = \frac{0.015 + 0.016 + 0.014}{3} = 0.015 \]
NCx must be \( \leq 0.2 \), otherwise, the test is invalid.

✓ Calculation of the PCx (Mean Absorbance of Positive Control)
Example:

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.846</td>
</tr>
<tr>
<td>2</td>
<td>0.902</td>
</tr>
</tbody>
</table>

\[ PCx = \frac{0.846 + 0.902}{2} = 0.874 \]
PCx must be \( \geq 0.5 \), otherwise, the test is invalid.

✓ Calculation of the P - N Value
P - N = PCx – NCx
Example: NCx = 0.015
PCx = 0.874
P - N = 0.874 - 0.015 = 0.859
P - N Value must be \( \geq 0.3 \), otherwise, the test is invalid.

✓ Calculation of the Cutoff Value
Cutoff Value = NCx + 0.025
Example: Cutoff Value = 0.015 + 0.025 = 0.040

✓ Calculation of the Retest Range
Retest Range = Cutoff Value ± 10%
Example: Cutoff Value = 0.040
Retest Range = (0.040 - 0.004) to (0.040 + 0.004) = 0.036 to 0.044
Validity of the Test Runs
1. NCx must be ≤ 0.2; otherwise, the test is invalid.
2. PCx must be ≥ 0.5, otherwise, the test is invalid.
3. P - N Value must be ≥ 0.3, otherwise, the test is invalid.

*Note: Negative Control: absorbance value must be less than or equal to 0.200 after subtracting the blank.*

Interpretation of Results
- Specimens with absorbance values less than (0.9 X Cutoff Value) are considered NON-REACTIVE and are considered NEGATIVE for Anti-HBs.
- Specimens with absorbance values greater than (1.1 X Cutoff Value) are considered REACTIVE and are considered POSITIVE for Anti-HBs.
- Specimens with absorbance value within the Retest Range (Cutoff Value ± 10%) shall be repeated in duplicate and interpreted as above. Specimens with any of the repeat results in the retest range are reported as "indeterminate." It is suggested to test follow-up samples for "indeterminate" results.

Performance Characteristics

Specificity
Results from the European Performance Evaluation for the Hepatitis B surface antigen Ab ELISA Kit - Reactivity of HBV Negative “Donor” and “Hospitalized persons” Specimens.

<table>
<thead>
<tr>
<th>Anti-HBs negative Sample</th>
<th>No. of sample</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unselected donor samples</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Hospitalized persons</td>
<td>134</td>
<td>134</td>
</tr>
<tr>
<td>Potential interfering samples</td>
<td>50</td>
<td>49</td>
</tr>
<tr>
<td>Total</td>
<td>584</td>
<td>583</td>
</tr>
</tbody>
</table>

Specificity = 583/584 = 99.83%

Analytical Sensitivity:
Detection limit determined using dilutions of Anti-HBs Standards.
The analytical sensitivity was determined to be 3.6 mIU/mL of anti-HBs for the Hepatitis B surface antigen Ab ELISA Kit assay using among others the PEI Anti-HBs Standard.
The S/CO for 10 mIU/mL of anti-HBs was about 1.60. For routine testing, it may be useful and practical to increase the cutoff value for the positive result to 1.6x standard/CO in order to achieve a cutoff of approximately 10 mIU/mL of Anti-HBs (“protective liter”).

Test Linearity using blood samples
Linearity was evaluated using two high-titer Anti-HBs-positive serum samples by diluting them throughout the measuring range of the assay and then around the cutoff level in narrow dilution steps.
The Hepatitis B surface antigen Ab ELISA Kit assay showed linear behavior on dilution between 3.6 and 240 mIU/mL.
✓ Sensitivity
Positive specimens/Specimens used to evaluate the sensitivity/Patients with HBV infection

<table>
<thead>
<tr>
<th>HBV infected individuals</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HBs positive samples</td>
<td>No. of samples</td>
<td>Positive results</td>
</tr>
<tr>
<td>Natural infected individuals</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>Hep B vaccinated individuals</td>
<td>111</td>
<td>111</td>
</tr>
<tr>
<td>Total</td>
<td>235</td>
<td>235</td>
</tr>
</tbody>
</table>

Sensitivity = 235/235 = 100%

✓ Evaluation of Precision
Accuracy: intra-run repeatability and inter-run reproducibility

The positive control of the Hepatitis B surface antigen Ab ELISA Kit (800 mIU/mL) and two serum samples with Anti-HBs levels just above cutoff and at medium level were tested in replicates of 20 in a single run over three days. The results were used to calculate the intra-run repeatability and inter-run reproducibility as presented in the following tables.

<table>
<thead>
<tr>
<th>Item tested</th>
<th>Sample size</th>
<th>precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-run</td>
<td>N = 20</td>
<td>CV ≤ 10.27%</td>
</tr>
<tr>
<td>Inter-run</td>
<td>N = 60</td>
<td>CV ≤ 7.11%</td>
</tr>
<tr>
<td>Serum #1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-run</td>
<td>N = 20</td>
<td>CV ≤ 7.57%</td>
</tr>
<tr>
<td>Inter-run</td>
<td>N = 60</td>
<td>CV ≤ 7.17%</td>
</tr>
<tr>
<td>Serum #2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-run</td>
<td>N = 20</td>
<td>CV ≤ 9.05%</td>
</tr>
<tr>
<td>Inter-run</td>
<td>N = 60</td>
<td>CV ≤ 7.71%</td>
</tr>
</tbody>
</table>
Resources

Troubleshooting

If the result cannot be reproduced, perform a preliminary troubleshooting by checking the possibilities listed below:

✓ Improper washing procedure.
✓ Contamination with positive specimen.
✓ Wrong volume of sample, conjugate or substrates.
✓ Contamination of the well rim with conjugate.
✓ Improper specimen, such as hemolyzed serum or plasma, specimen containing sediments and specimen not thoroughly mixed before use.
✓ Wrong incubation time or temperature.
✓ Obstructed or partial obstructed washer aspirate/dispense head and needles.
✓ Insufficient aspiration.

References

### Plate Layout

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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