PROCEDURAL CAUTIONS AND WARNINGS

1. All the reagents within the kit are calibrated for the direct determination of CRP in human serum. The kit is not calibrated for the determination of CRP in saliva, plasma or other specimens of human or animal origin.

2. Do not use grossly hemolyzed, grossly lipemic, icteric or otherwise discolored sera.

3. Any samples or control sera containing azide or thimerosal should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.

4. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.

5. The results obtained with this kit should never be used as the sole basis for clinical diagnosis. For example, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/contact with infectious agents.

6. Some individuals may have antibodies to mouse protein that can possibly interfere in this assay. Therefore, the results from any patients who have received preparation labelled tube and allow it to clot. Centrifuge and carefully remove any bubbles before performing the reading step.

7. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated with OD values for the controls do not reflect established ranges.

8. Do not dilute the standards and controls, they are ready for use.

9. Control materials or serum pools should be included in every run and fail within established confidence limits.

10. The substrate solution (TMB) is sensitive to light and should not be used when dispensed.

11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.

12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.

13. Do not mix various lots number of kits components within a test to reduce the variation expected beyond existing expiration date printed on the label.

14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of CRP in human serum. The kit is not calibrated for the determination of CRP in saliva, plasma or other specimens of human or animal origin.

2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored sera.

3. Some samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.

4. Only calibrator A may be used to dilute any high serum samples.

5. The results obtained with this kit should never be used as the sole basis for clinical diagnosis. For example, the occurrence of heterogeneous antibodies in patients regularly exposed to animals/contact with infectious agents. As a result, increased serum CRP concentration is positively associated with the risk of future coronary events.

PROCEDURAL CAUTIONS AND WARNINGS

1. Users should have a thorough understanding of the protocol for the successful use of this kit. Reliable performance can only be attained by strictly following the instructions provided.

2. Control materials or assay pools should be included in every run at a high and low level for assessing the reliability of results.

3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.

4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling reagents and human specimens.

5. All kit reagents and specimens should be kept at room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.

6. A calibrator curve must be established for every run.

7. The controls should be included in every run and fail within established confidence limits.

8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated with OD values for the controls do not reflect established ranges.

9. When reading the microtitre plate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

10. The substrate solution (TMB) is sensitive to light and Should not be used when dispensed.

11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.

12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.

13. Do not mix various lots number of kits components within a test to reduce the variation expected beyond existing expiration date printed on the label.

14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

REF: CAN-CRP-4360
Version: 6.0
Effective: September 13, 2018

REFERENCES

1. High sensitivity C-Reactive Protein (hs-CRP) ELISA:
   EU - CAN - CRP-4360

2. Diagnostics Biochem Canada Inc.
   Manufacturing Innovative IVD for the World
   Division of Biochem Scientific Corporation

3. www.diagnosticsbiochem.com

4. Effective: September 13, 2018

5. Version: 6.0

6. 1/2
ASSAY PROCEDURE

Specimen Pretreatment
Dilute 1:20 With Calibrator A Before Use.

1. Prepare working solutions of the anti-CRP-HRP conjugate and wash buffer.
2. Remove the required amount of well strips. Seal the bag and return any unused strips to the refrigerator.
3. Pipette 20 µL of each calibrator, control and diluted specimen sample into correspondingly labelled wells in duplicate.
4. Pipette 200 µL of assay buffer into each well. (We recommend using a multichannel pipette.)
5. Incubate on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.
6. Wash the wells 3 times with 300 µL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
7. Pipette 100 µL of the conjugate working solution into each well. (We recommend using a multichannel pipette.)
8. Incubate on a plate shaker (approximately 200 rpm) for 15 minutes at room temperature.
9. Wash the wells again in the same manner as step 6.
10. Pipette 100 µL of TMB substrate into each well at timed intervals.
11. Incubate on a plate shaker for 10–15 minutes at room temperature (or until calibrator F attains dark blue colour for desired OD).
12. Pipette 50 µL of stopping solution into each well at within 20 minutes after addition of the stopping solution.

TYPICAL CURVATURE

Sample curve only. Do not use to calculate results.

INTER-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Apparent CRP Value (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Albumin</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Human Globulin</td>
<td>Not Detected</td>
</tr>
</tbody>
</table>

SPECIFICITY (CROSS-REACTIVITY)

The specificity of the hs-CRP ELISA kit was determined by measuring the apparent CRP value of spiked samples with the following compounds:

<table>
<thead>
<tr>
<th>Substance</th>
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</tr>
</thead>
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<tr>
<td>Human Albumin</td>
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</tr>
<tr>
<td>Human Globulin</td>
<td>Not Detected</td>
</tr>
</tbody>
</table>

HIGH DOSE HOOK EFFECT

The hs-CRP ELISA kit did not experience a high dose hook effect.

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) plus 2 SD. Therefore, the specificity of the DBC hs-CRP ELISA kit is 10 ng/mL.

SPECIFICITY (CROSS-REACTIVITY)

The specificity of the hs-CRP ELISA kit was determined by measuring the apparent CRP value of spiked samples with the following compounds:

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</tr>
<tr>
<td>Human Globulin</td>
<td>Not Detected</td>
</tr>
</tbody>
</table>

TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

<table>
<thead>
<tr>
<th>Calibrator OD</th>
<th>OD 1</th>
<th>OD 2</th>
<th>Mean OD</th>
<th>Value (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.055</td>
<td>0.053</td>
<td>0.054</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>0.105</td>
<td>0.103</td>
<td>0.104</td>
<td>100</td>
</tr>
<tr>
<td>C</td>
<td>0.271</td>
<td>0.276</td>
<td>0.274</td>
<td>400</td>
</tr>
<tr>
<td>D</td>
<td>0.607</td>
<td>0.633</td>
<td>0.620</td>
<td>1000</td>
</tr>
<tr>
<td>E</td>
<td>7.36</td>
<td>8.19</td>
<td>7.73</td>
<td>4000</td>
</tr>
<tr>
<td>F</td>
<td>2.829</td>
<td>2.827</td>
<td>2.828</td>
<td>10,000</td>
</tr>
<tr>
<td>Unknown</td>
<td>1.035</td>
<td>1.048</td>
<td>1.042</td>
<td>1737</td>
</tr>
</tbody>
</table>

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample Mean</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1.03</td>
<td>15.2</td>
</tr>
<tr>
<td>2</td>
<td>762</td>
<td>38.4</td>
</tr>
<tr>
<td>3</td>
<td>8437</td>
<td>70.6</td>
</tr>
</tbody>
</table>

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample Mean</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>227.0</td>
<td>22.4</td>
<td>9.9</td>
</tr>
<tr>
<td>1022.2</td>
<td>97.2</td>
<td>9.5</td>
</tr>
<tr>
<td>6791.8</td>
<td>685.8</td>
<td>8.7</td>
</tr>
</tbody>
</table>

REFERENCES


DBC-Diagnostics Biochem Canada Inc.
384 Neptune Crescent
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Fax: (519) 681-8734
e-mail: ddc@dbc-labs.com
dbc-labs.com
An ISO 13485 Registered Company

EMERGO EUROPE
Pinsессегрот 28
144 AP The Hague
The Netherlands

SYMBOLS

Legal Manufacturer
Authorized Representative

LOT
Lot number
# Dilute 1:20 before use