VALIDATION OF A NEW CANINE-SPECIFIC DRY CHEMISTRY ASSAY FOR THE QUANTIFICATION OF CANINE C-REACTIVE PROTEIN

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BACKGROUND

C-reactive protein (CRP) is a major positive acute phase protein (APP) in dogs, produced mainly in the liver in response to increased concentrations of pro-inflammatory cytokines1 in a wide variety of diseases in dogs, such as: infectious and immune-mediated diseases or neoplasia1. Therefore, CRP measurement is one of the most sensitive tests to detect inflammation in this species that can be done in clinical practice2.

OBJECTIVE

The objective of the current study was to validate the use of a new canine-specific dry chemistry assay for in-house CRP measurement [FUJI DRI CHEM SLIDE vc-CRP-P, FUJIFILM®] (Figure 1). For this purpose, an analytical validation was conducted, as well as the evaluation of the assay to detect different serum CRP concentrations between healthy and dogs with inflammation.

MATERIAL AND METHODS

Samples. 15 canine serum samples were selected to create three pools (5 serum samples each one):
• 1 pool with high CRP concentration (100 mg/L)
• 1 pool with medium CRP concentration (50 mg/L)
• 1 pool with low CRP concentration (10 mg/L)

Analytical validation. The following parameters were determined:
• Precision:
  - Intra-assay precision was determined by measuring each of the pools 5 times in the same analytical series (Table 1).
  - Inter-assay precision was done measuring each pool in 5 different days (Table 1).
• Sensitivity:
  - Limit of detection (LD) was determined from 12 replicate measurements of zero standard (assay diluent) as mean value +2 SD (Table 2).
  - Lower limit of quantification (LLOQ) was the lowest CRP concentration that could be measured which the coefficient of variation (CV) of 5 repeated determinations below 20% (Table 2).

Clinical validation. 36 canine serum samples (20 with inflammatory diseases and 16 healthy) was selected from our database (Figure 3).

RESULTS

Analytical validation

<table>
<thead>
<tr>
<th>Pool</th>
<th>Intra-assay</th>
<th>Inter-assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>2.0%</td>
<td>9.45%</td>
</tr>
<tr>
<td>Medium</td>
<td>2.64%</td>
<td>3.55%</td>
</tr>
<tr>
<td>Low</td>
<td>7.9%</td>
<td>14.4%</td>
</tr>
</tbody>
</table>

Table 1. Coefficient of variation (%) for the intra and inter-assay.

<table>
<thead>
<tr>
<th>Limit</th>
<th>CRP (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD</td>
<td>3</td>
</tr>
<tr>
<td>LLOQ</td>
<td>10.4</td>
</tr>
</tbody>
</table>

Table 2. Limit of detection (LD) and lower limit of quantification (LLOQ).

Clinical validation

DISCUSSION AND CONCLUSIONS

The precision study showed that the CVs were acceptable for the pools with high and medium CRP concentrations. Although high CVs were observed with low CRP concentrations, it has been observed that the maximum value of CRP to consider not inflammation can be established at 12 mg/L,3 well above of the LLOQ detected in this assay and, therefore, would not have a major implication in the clinical decision in order to rule in or rule out active inflammation or sepsis.

Analytical results provided in this study indicate that the method can measure high and medium CRP concentrations with adequate precision and accuracy. In addition, from the clinical point of view, the assay was able to detect changes in CRP levels in dogs with inflammatory diseases compared with healthy dogs.

References