Validation of spectrophotometric assays for serum paraoxonase type-1 measurement in dogs

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Objective—To evaluate and validate 3 spectrophotometric assays for measuring serum activity of paraoxonase type-1 (PON1), an enzyme associated with high-density lipoproteins, in dogs.

Animals—22 healthy adult dogs and 10 dogs with eccentrocytosis.

Procedures—2 methods were adapted for use in 96-well microplates with phenyl acetate and 5-thiobutyl butyrolactonase as substrates, and 1 was adapted for use in an automated analyzer with p-nitrophenyl acetate as substrate. Blood samples were collected from all dogs, serum was harvested, and serum PON1 activity was measured with each method.

Results—Imprecision was low for all 3 methods, with the exception of interassay imprecision for 5-thiobutyl butyrolactonase, and results were linear across serial sample dilutions. The 3 methods were able to detect low PON1 activity when EDTA was used for blood sample collection, yielded lower PON1 values in sick dogs with eccentrocytosis than in healthy dogs, and yielded highly correlated results.

Conclusions and Clinical Relevance—The methods described here may allow a wider use of PON1 activity as a biomarker of oxidative stress in dogs in clinical and research settings. Results of each method were robust and precise (with the exception of the interassay values for the lactonase method), and the methods were easy to set up in a laboratory. (Am J Vet Res 2012;73:34–41)
Materials and Methods

Animals—Sixteen 6- to 8-year-old healthy adult dogs (5 sexually intact females, 3 spayed females, 7 sexually intact males, and 1 castrated male) were evaluated at San Marco Veterinary Hospital and included in the study. Breeds represented included mixed (n = 9), Golden Retriever (2), American Cocker Spaniel (2), Yorkshire Terrier (2), and German Shepherd Dog (1). All dogs had no evident acute or chronic disease. Results of physical and clinical examinations were unremarkable, as were results of routine hematologic and serum biochemical analyses. Also included were 6 healthy 6- to 8-year-old Beagles (3 sexually intact females and 3 sexually intact males) from the Murcia Animal Resources Centres.

In addition to the healthy dogs, ten 4- to 15-year old sick dogs (4 sexually intact females, 1 spayed female, 3 sexually intact males, and 2 castrated males) were included in which centrocystosis (an indicator of oxidative cell damage) was diagnosed at the San Marco Veterinary Hospital during 2009. These dogs were of various breeds and had a broad range of hematologic abnormalities. Diagnoses were as follows: diabetes mellitus (n = 3), onion intoxication (2), acute leukemia (2), rodenticide intoxication (1), hemangio (1), and brain tumor (1).

All procedures involving the Beagles from Murcia Animal Resources Centres were performed in accordance with the animal care guidelines of the University of Murcia. Consent for study participation was obtained from owners of the 26 dogs that were evaluated at San Marco Veterinary Hospital.

Sample collection and processing—After food was withheld from the dogs for at least 12 hours overnight, blood samples were collected from each via jugular or lateral saphenous venipuncture into tubes containing EDTA for hematologic analysis and tubes with clotting accelerator for serum biochemical analysis. Samples were centrifuged at 2,000 × g for 10 minutes at room temperature (20° to 22°C), and serum was harvested. The CBCs and serum biochemical analyses were performed with automatic analyzers.

Total antioxidant capacity was determined as described elsewhere. The method used is based on 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) decolorization by antioxidants according to their concentrations and antioxidant capacities. The color change is measured as a change in light absorbance at 660 nm. For the process, an automated analyzer was used, and the assay was calibrated with 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

Biochemical measurement of PON1 activity—Serum lactonase activity was analyzed by measuring the hydrolysis of TBBL (assay A). The method involves use of a chromogenic lactone that structurally resembles the proposed natural lipolactone substrates. Serum samples were prepared in sample buffer consisting of 50mM Tris and 1mM CaCl₂ (pH, 8.0) in a 20-fold dilution. One microliter of 100mM 5,5′-dithio-bis-2-nitrobenzoic acid in dimethyl sulfoxide, 45 µL of 4% acetonitrile solution in sample buffer, 5 µL of diluted serum, and 50 µL of sample buffer then were added to the wells of a 96-well microplate. Finally, 100 µL of freshly made substrate buffer containing 0.4mM TBBL solution in sample buffer was added to the wells to initiate the reaction. Two minutes after TBBL addition, the reaction was monitored at 412 nm in an automated microplate reader at 37°C. The nonenzymatic hydrolysis of TBBL, which was based on the hydrolysis rate in the absence of serum, was subtracted from the total hydrolysis rate. Lactonase activity was expressed as units per milliliter of serum, in which 1 unit equals 1 µmol of TBBL hydrolyzed/min. The molar extinction coefficient used to calculate the rate of hydrolysis was 7,000 M⁻¹ cm⁻¹. A path-length correction was applied for the use of microtiter plates.

Serum arylesterase activity was analyzed by measuring the hydrolysis of phenyl acetate into phenol (assay B) as described elsewhere. Serum samples were prepared in sample buffer consisting of 20mM Tris and 1mM CaCl₂ (pH, 8.0) in a 40-fold dilution. Five microliters of diluted serum was added to 200 µL of freshly made substrate buffer containing 20mM Tris, 1mM CaCl₂, and 1mM phenyl acetate (pH, 8.0). The reaction was monitored on a microtiter plate at 260 nm in an automated microplate reader at 37°C. The nonenzymatic hydrolysis of phenyl acetate, which was based on the hydrolysis rate in the absence of serum, was subtracted from the total hydrolysis rate. Arylesterase activity is expressed as units per milliliter of serum, in which 1 U equals 1 µmol of phenyl acetate hydrolyzed/min. The molar extinction coefficient used to calculate the rate of hydrolysis was 1,310 M⁻¹ cm⁻¹. A path-length correction was applied for the use of microtiter plates.

Serum arylesterase activity was also analyzed by measuring the hydrolysis of p-nitrophenyl acetate to p-nitrophenol as described elsewhere, but a modification to remove substrate from the working reagent buffer and prepare it in water as a separate starting reagent that remained colorless (assay C). This modification was made because p-nitrophenylacetate is subject to considerable spontaneous hydrolysis in the reagent buffer system originally described, and this hydrolysis was grossly apparent in the yellow color of the reagent. The starting reagent was added to initiate the kinetic reaction.

Because p-nitrophenyl acetate is insoluble in water, 63 mg of this compound was dissolved in 10 mL of methanol and stored at 2° to 8°C. In our experience, this stock solution can be kept for approximately 1 week with only a small increase in free p-nitrophenol. Afterward, 1 mL of this solution was slowly added to 20 mL of distilled water with strong agitation to prevent precipitation. The aqueous solution was freshly prepared each day.

To perform assay C, serum samples were each mixed with 307 µL of buffer containing 50mM Tris and 1mM CaCl₂ (pH, 8.0) and then freshly made substrate containing 2.5mM p-nitrophenyl acetate in distilled water was added. After 100 seconds, the reaction was monitored at 405 nm at 37°C for 210 seconds in an automated biochemistry analyzer. The nonenzymatic hydrolysis of phenyl acetate, which was based on the hydrolysis rate in the absence of serum, was subtracted from the total hydrolysis rate. The activity was expressed as units per milliliter of serum, in which 1 U...
solution was serially diluted with sample buffer, and
ing assay buffer to create a stock solution. The stock
sions with a triglycerides concentration of 200 g/L was
sure PON1 activity with the 3 assays.
lysis (4 and 8 g/L). Prepared samples were used to mea-
concentration was achieved by adding 10
trations were 8, 4, 2, 1, 0.5, and 0.0 g/L. The 0.0 g/L
ples of canine serum. The final hemoglobin concen-
10
nine serum. Homogeneity was achieved by mixing with
Beagles were mixed with various concentrations of
expression as described,17 and each
preparation was run in duplicate. For hemolysis inves-
tigation, a fresh hemolysate was prepared by addition
of distilled water to packed, saline solution–washed
erythrocytes from 1 Beagle. Hemoglobin concentration in he-
of hydrolysis was 14,000 M–1 cm–1.
The molar extinction coefficient used to calculate the rate
of hydrolysis was 14,000 M–1 cm–1.
Assay validation—For analytic validation of the
3 methods used for PON1 activity measurement, the
following characteristics were calculated: precision,
intra-assay CV, accuracy, and limit of detection. Two
pools of sera with different PON1 activities were prepared
from the group of healthy dogs and the group of
dogs with eccentricytosis and used for precision
evaluations. To determine interassay precision, these
pools were divided in aliquots and stored in plastic
vials at –20°C until analysis. On the day of analysis, samples were brought to room temperature prior to
PON1 measurement. Intra-assay CV was calculated after analysis of the 2 serum pools 6 times in 1 assay run. Interassay CV was determined by analyzing the same
pools in 6 separate runs performed on different
days.
Because of the lack of a criterion-referenced stan-
dard or commercially available certified reference mate-
rial for canine PON activity, assay accuracy was evalu-
ed indirectly through assessment of dilutional linearity. For this process, 2 canine serum samples were serially
diluted with saline (0.9% NaCl) solution and analyzed by
use of the procedures previously described. Limit of
detection was calculated on the basis of data from 20
replicate determinations of the zero standard (buffer of
assays) as mean value plus 2 SDs.
To determine whether results of the 3 assays were
correlated, serum samples from all study dogs were
evaluated with each assay. All samples used for repeti-
tive analysis were frozen in aliquots, and to avoid possi-
bile changes due to repetitive thawing and freezing, only vials needed for each run were used.
Effects of hemolysis and lipemia—To investigate
effect of hemolysis and lipemia on results of the 3
assays for PON1 activity, 3 serum samples from 3 healthy
Beagles were mixed with various concentrations of
hemoglobin or lipid solution as described,17 and each
preparation was run in duplicate. For hemolysis inves-
tigation, a fresh hemolysate was prepared by addition
of distilled water to packed, saline solution–washed
erythrocytes from 1 Beagle. Hemoglobin concentration in he-
emolysate was determined by use of a veterinary animal
blood cell counterb and adjusted to 200 g/L by add-
ing assay buffer to create a stock solution. The stock
solution was serially diluted with sample buffer, and
10 µL of each dilution was added to three 90-µL sam-
ples of canine serum. The final hemoglobin concen-
trations were 8, 4, 2, 1, 0.5, and 0.0 g/L. The 0.0 g/L
concentration was achieved by adding 10 µL of sample
diluent buffer. These hemoglobin concentrations were
intended to correspond to slight hemolysis (0.5 g/L),
moderate hemolysis (1 and 2 g/L), and marked hemo-
lysis (4 and 8 g/L). Prepared samples were used to mea-
sure PON1 activity with the 3 assays.
For the lipids investigation, commercial fat emul-
sionc with a triglycerides concentration of 200 g/L was
serially diluted with sample diluent buffer and 10 µL of
each dilution was added to two 90-µL samples of ca-
nine serum. Homogeneity was achieved by mixing with
a vortex device. The final triglycerides concentrations
were 5, 2.5, 1.25, 0.625, and 0.3125 g/L, which were
intended to correspond to slight lipemia (0.3125 and
0.625 g/L), moderate lipemia (1.25 and 2.5 g/L), and
marked lipemia (5 g/L). Prepared samples were used to
measure PON1 activity with the 3 assays.
Effects of EDTA—Blood samples (8 mL each) from 6 Beagles were obtained via jugular venipuncture by
use of a disposable syringe and 21-gauge needle. Ali-
quot of the samples were placed in 2 types of tubes: one
containing 0.072 mL of 7.3% tripotassium methy-
lenediaminetetraacetic acid (EDTA K3)7 for EDTA-
treated plasma and the other containing clot activator
(5-mL tubea) for serum. All samples were centrifuged at
2,000 × g for 10 minutes, and serum and EDTA-treated
plasma were immediately separated prior to analysis on
the same day. The interval between collection and cen-
trifugation was approximately 30 minutes. All plasma
and serum samples were analyzed by use of the 3 study
methods to measure PON1 activity.
Statistical analysis—Arithmetic means, medians, and
intra-assay and interassay CVs were calculated by
use of routine descriptive statistical procedures and
computer software.8 Dilutional linearity was evaluated
through ordinary linear regression analysis in which
the measured activities of PON1 were compared with
the expected activities. Correlation among results ob-
tained with the 3 methods was assessed by use of linear
regression. Interferograms were prepared to show the
differences in PON1 activities when hemoglobin or tri-
glycerides were added. The influence of hemoglobin or
triglycerides on PON1 activity was investigated by use
of 1-way ANOVA and Dunnett posttests. Comparisons
of the results for serum and EDTA-treated plasma were
made by use of a Student t test for repeated measure-
ments. A Student t test was used to evaluate the differ-
ence of TAC and PON1 activities between healthy dogs
and dogs with eccentricytosis. The correlation between
PON1 activity and TAC was evaluated by calculation of the
Spearman correlation coefficient (ρ). Values of P <
0.05 were considered significant for all analyses.
Results
Assays—For assay A (serum lactonase activity by
use of microplates), intra-assay and interassay CV ran-
ges were 5.5% to 6.7% and 11.3% to 17.7%, respectively
(Table 1). Serial dilution of 2 serum samples resulted in
linear regression equations with correlation coefficients
close to 1 (r = 0.997 and 0.953; Figure 1). The assay
detection limit was 1.0 U/mL (mean ± SD, 0.4 ± 0.2 U/
ml). For assay B, intra-assay and interassay CV ranges
were 4.0% to 4.8% and 5.4% to 9.5%, respectively. Seri-
al dilution of 2 serum samples resulted in linear regres-
sion equations with correlation coefficients close to 1 (r
= 0.997 and 0.996). The assay detection limit was 9.1
U/mL (mean ± SD, 1.8 ± 2.5 U/mL). For assay C, intra-
and interassay CV ranges were 4.5% to 5.6% and 7.3%
to 8.2%. Serial dilution of 2 serum samples resulted in
linear regression equations with correlation coefficients
close to 1 (r = 0.997 and 0.995). The assay detection
limit was 0.6 U/mL (mean ± SD, 0.49 ± 0.05 U/mL).
When results for 38 serum samples analyzed with the 3 assays were used, linear regression analysis revealed significant correlations between the results of assays A and B \((P < 0.001; r = 0.852)\), A and C \((P < 0.001; r = 0.850)\), and B and C \((P < 0.001; r = 0.870)\).

<table>
<thead>
<tr>
<th>Assay</th>
<th>Comparison</th>
<th>Pool</th>
<th>Mean ± SD activity (U/mL)</th>
<th>Mean CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Intra-assay</td>
<td>1</td>
<td>4.3 ± 0.2</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>Interassay</td>
<td>1</td>
<td>2.0 ± 0.9</td>
<td>17.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>4.4 ± 0.5</td>
<td>11.3</td>
</tr>
<tr>
<td>B</td>
<td>Intra-assay</td>
<td>1</td>
<td>20.1 ± 0.8</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Interassay</td>
<td>1</td>
<td>21.7 ± 1.2</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>17.5 ± 1.7</td>
<td>9.5</td>
</tr>
<tr>
<td>C</td>
<td>Intra-assay</td>
<td>1</td>
<td>5.1 ± 0.2</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>Interassay</td>
<td>1</td>
<td>5.5 ± 0.4</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>3.1 ± 0.3</td>
<td>8.2</td>
</tr>
</tbody>
</table>

Effects of hemolysis and lipemia on assay results—For assay A, a significant decrease in PON1 activities was evident when hemoglobin was added to the serum samples at a concentration of 8 g/L or when triglycerides were added at a concentration of 5 g/L (Figures 2 and 3). For assay B, a significant decrease in PON1 activities appeared when triglycerides at a concentration of 5 g/L were added to the samples. No substantial interference in PON1 activities by hemoglobin was evident at concentrations < 8 g/L. For assay C, a significant decrease in PON1 activities was evident when triglycerides at a concentration of ≥ 1.25 g/L were added to the samples. No significant interference by hemoglobin was evident at concentrations < 8 g/L.

Effects of EDTA on assay results—Results obtained when serum and EDTA-treated plasma samples were evaluated by use of the 3 methods were summarized (Table 2). Significant \((P < 0.001)\) decreases in PON1 activity were detected in EDTA-treated plasma samples versus serum samples for each assay.

Assay discrimination—Activities of PON1 as well as TAC in dogs with eccentrocytosis were significantly lower than those in healthy dogs when analyzed with
the 3 methods (Table 3). The Spearman correlation test revealed a positive, significant correlation between TACs and results of assays A ($\rho = 0.64; P = 0.002$), B ($\rho = 0.49; P = 0.027$), and C ($\rho = 0.48; P = 0.033$).

**Discussion**

In the study reported here, 3 spectrophotometric methods for measurement of PON1 activity were evaluated and validated. A different substrate was used in each assay: TBBL for lactonase activity and p-nitrophenyl acetate and phenyl acetate for arylesterase activity. Paraoxon is a highly toxic and unstable compound that is unsuitable for routine high-throughput use, so measurements of paraoxonase activity of PON1 were not included in the present study. In humans, arylesterase or lactonase activities are less variable between subjects than is paraoxonase activity, which is influenced by genetic variation.\(^2\),\(^6\),\(^10\)

Although PON1 was long considered to be an arylesterase and paraoxonase and its activity was measured accordingly, it recently became apparent that PON1 can catalyze the hydrolysis and formation of various lactones. Lactonase activity is the only activity shared by all other members of the PON family, some of which have no paraoxonase or arylesterase activity.\(^18\) Some investigators have consequently postulated that use of phenyl acetate or paraoxon would not have physiologic relevance and have suggested that assays of PON1 activity should mainly address the lactonase activity.\(^12\) However, because the substrate for lactonase activity measurements is not widely available and a correlation between arylesterase and lactonase activities has been reported for humans,\(^7\) we aimed to validate assays not only for lactonase activity but also for arylesterase activity.

![Figure 2](image1.png)

**Figure 2**—Interferograms corresponding to the effect of hemoglobin concentration on PON1 activity as measured by use of 3 assays: lactonase activity via measurement of TBBL hydrolysis (A), arylesterase activity via measurement of phenyl acetate hydrolysis (B), and arylesterase activity via measurement of p-nitrophenyl acetate hydrolysis (C). Values for each given concentration (Vf) are reported as a percentage of the original value (Vo).

![Figure 3](image2.png)

**Figure 3**—Interferograms corresponding to the effect of triglycerides concentration on PON1 activity as measured by use of the assays in Figure 2. See Figure 2 for remainder of key.
had an interassay CV of 17.7%, which is similar to values reported when the method is used in humans. Ideally, when lactonase activity is the analyte of interest, serum samples should be analyzed in the same batch to avoid these interassay variations. Perhaps because its suitability for automation diminishes analytic errors, p-nitrophenyl acetate is reportedly more sensitive than other substrates for detecting variations in PON1 activity. Moreover, p-nitrophenyl acetate is a nontoxic ester that is less volatile than lactone and thus would be more suitable for routine measurement of PON1 activity.

Hemolysis did not result in substantial interferences in the arylesterase assays, and only a significant decrease in serum PON1 activity was evident with the lactonase method at a high hemoglobin concentration (8 g/L). This finding is similar to findings in humans regarding the lactonase assay; in humans, results are not affected by hemolysis when hemoglobin values of < 6 g/L are present, indicating hemolysis has no influence on PON1 assay results unless it is severe. In lipemic serum samples, significant decreases in PON1 activities were observed with increasing triglycerides concentrations with all 3 methods. A negative correlation between serum PON1 activity and triglycerides has been reported for humans. In addition, lipemic samples can interfere with many clinical chemistry tests through various mechanisms, the most common of which is the scattering of light rays by the lipids (mainly chylomicrons and very low-density lipoproteins).

Results of all the methods used in the present study were highly correlated. In humans, similarly significant correlations exist between results of arylesterase and lactonase assays. However, results of PON1 activity testing in humans with certain diseases can vary depending on the substrate used, so the simultaneous use of at least 2 substrates to measure PON1 activity is recommended to improve the reliability of the results.

To test the ability of the methods to detect and differentiate between PON1 activities, we performed in vitro assays to assess the influence of EDTA and oxidative agent illness (eccentrocytosis) on PON1 activity. Reportedly, PON1 has an absolute requirement for Ca²⁺ for activity and stability, so use of EDTA can inactivate PON1. Our results are in agreement with previous findings that a significant decrease of PON1 activity occurs when EDTA-treated plasma is used instead of serum.

Eccentrocytes are erythrocytes in which hemoglobin is concentrated on 1 side of the cell, leaving a pale or clear eccentric space. These cells are associated with oxidative injury of erythrocytes in humans and animals. Increased amounts of endogenous oxidants are generated in various disorders, such as inflammation, neoplasia, and diabetes, or when reducing pathways in erythrocytes are defective, as occurs with glucose-6-phosphate dehydrogenase and flavin adenine dinucleotide deficiencies. In the present study, dogs with eccentrocytosis had lower TAC values than healthy dogs, indicating oxidative damage. The decrease of PON1 in dogs with eccentrocytosis in the present study indicates that the validated methods were able to detect lower PON1 values associated with oxidative stress. As

Table 2—Activities of PON1 in serum and EDTA-treated plasma sample aliquots as measured by use of 3 assays.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sample</th>
<th>Activity in serum (U/mL)</th>
<th>Activity in plasma with EDTA (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>4.59</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.36</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.23</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3.67</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.71</td>
<td>1.96</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>4.36</td>
<td>0.68</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>4.32 ± 0.36</td>
<td>0.96 ± 0.60*</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>21.28</td>
<td>9.10</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>21.28</td>
<td>9.10</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>18.78</td>
<td>9.10</td>
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<td>18.78</td>
<td>9.10</td>
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<td>5</td>
<td>18.03</td>
<td>11.27</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>15.02</td>
<td>9.10</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>19.20 ± 2.33</td>
<td>9.46 ± 0.89*</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>6.51</td>
<td>1.87</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.83</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td>3</td>
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<td>4</td>
<td>4.92</td>
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<td>5</td>
<td>5.63</td>
<td>2.38</td>
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<td></td>
<td>6</td>
<td>4.69</td>
<td>1.17</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>5.02 ± 0.68</td>
<td>1.96 ± 1.22*</td>
<td></td>
</tr>
</tbody>
</table>

*Value differs significantly (P < 0.001) from value for serum.

Table 3—Mean ± SD PON1 activity determined by use of 3 assays and TAC in 10 dogs with eccentrocytosis and 22 healthy dogs.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Dogs with eccentrocytosis</th>
<th>Healthy dogs</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON1 activity (U/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assay A</td>
<td>4.10 ± 1.22</td>
<td>4.82 ± 0.50</td>
<td>0.048</td>
</tr>
<tr>
<td>Assay B</td>
<td>14.97 ± 6.13</td>
<td>20.00 ± 2.37</td>
<td>0.023</td>
</tr>
<tr>
<td>Assay C</td>
<td>4.82 ± 1.44</td>
<td>6.31 ± 0.68</td>
<td>0.010</td>
</tr>
<tr>
<td>TAC (mmol)</td>
<td>0.35 ± 0.29</td>
<td>0.66 ± 0.18</td>
<td>0.011</td>
</tr>
</tbody>
</table>

A value of P < 0.05 was considered significant.

Initially, we intended to fully automate all 3 methods to improve the assay precision and decrease the time needed to analyze PON1 activity in each sample. However, the assays that involved phenylacetate and lactonase were validated in a 96-well ELISA plate format because the absorbance of phenylacetate at < 340 nm and instability of lactonase in aqueous solutions precluded its adaptation to automated analyzers. Although these assays were not fully automated, analysis involving a 96-well ELISA plate is fast and enables simultaneous processing of large batches of serum samples. In addition, spectrophotometric readers for 96-well ELISA plates are widely available in biochemical laboratories, so this technology could be suitable for use in veterinary diagnostic laboratories.

The validation results showed that the 3 methods were practical, rapid, and allowed evaluation of multiple samples at the same time; they also required a low volume of sample. These characteristics are also important if the measurement is to be included in a battery of several tests or in situations in which sample volume might be limited. Both arylesterase assays had CVs < 10% in all assessed situations, which is similar to assay validation findings in humans. The low CVs suggest a good assay reproducibility and support their suitability for use in other studies. However, the lactonase method

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AJVR, Vol 73, No. 1, January 2012

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has been reported for humans, a significant correlation was detected between PON1 activities and TACs. These data indicate that the PON1 methods described here can be used to evaluate oxidative status in dogs.

The 3 assays described here will allow a wider use of PON1 activity as a biomarker of oxidative stress in dogs because the assays were robust, precise (with the exception of the interassay values for lactonase method), and easy to set up in laboratories. As in human medicine, these assays could allow additional research into PON1 behavior in situations that can alter oxidative status, such as sepsis and renal or chronic liver disease in dogs; eventually, such studies could lead to applications already described for humans, such as the use of PON1 as a prognostic marker of disease progress and recovery in patients with chronic liver disease or sepsis.

References


