**PROCEDURE**
Specimens should be collected by approved veterinary techniques. Allow the blood to clot, and centrifuge to collect serum. Use serum within 72 hours, or freeze until ready to assay. Avoid repeated freezing and thawing of samples.

Add reference standards by carefully pipetting 3µl of each into the first wells. Be sure to lift the pipette off of the well bottom once the filling process has begun.

Pipette 3µl of the specimen(s) to be tested into the remaining well(s).

Replace the cover firmly on the plate and leave undisturbed, rightside up at room temperature for 18-24 hours outside of the mylar pouch.

After 18-24 hours, the diameters of the rings may be read and a standard curve established. Measure each diameter either directly off the plate or by using a comparator. Read each diameter in mm. If all wells are not used, replace the lid securely and store at 4-8 °C inverted in the mylar pouch provided.

Plot the diameters of the reference standards versus the concentration of the reference standards as indicated on the supplied graph paper. Draw a standard curve. Alternatively, a calculator with the capability of establishing a regression line may be used to determine the concentration of the unknowns. Specimens with diameters beyond the range of the standard curve may be retested either by redilution if the rings are too large or by concentration if the rings are too small.

**Interpretation**
Information sheets regarding immunodeficiencies and immunoproliferative disorders of horses, cows and dogs are available through VMRD, Inc.

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**SRID Kit**
Single Radial Immunodiffusion
For Veterinary Research Use Only

**PRINCIPLE**
The technique of single radial immunodiffusion (SRID) is the most widely used method for quantitative determination of classes of immunoglobulins and other serum and plasma proteins. This technique combines rapid and easy sample application with a high degree of accuracy and reproducibility.

The method is derived primarily from the works of Fahey, and of Mancini. Antiserum specific for the protein to be measured is incorporated into agarose gel. The sample antigen diffuses into the gel containing the antibody, and a ring of precipitation forms that is proportional in size to the concentration of the antigen. A linear relationship exists between the diameter of the ring and the concentration of the antigen when plotted on semi-log graph paper. This method is time and temperature dependent.

**LIMITATIONS**
Results are limited by the assay range of the kit, although dilution of the specimen to be tested will extend the range. When diameters greater than the highest reference standard occur, dilute the sample and rerun the assay. If the diameter of the ring is too small, the sample may be concentrated. VMRD supplies different ranges of test kits to accommodate your testing needs. Please see immunocheck at www.vmrd.com

**KIT CONTENTS**
1. Ready-to-use single radial immunodiffusion (SRID) plates containing monospecific antisera in buffered agarose
2. Four reference standards (preserved with 0.09% sodium azide)
3. 3 µl precision pipette
4. Instruction sheet with graph paper.

**MATERIALS NEEDED**
All the materials required for this test are included in the kit. If dilutions of sample are necessary use glass or plastic tubes.

**STORAGE AND PRECAUTIONS**
Store plates in an inverted position at 4-8 °C. **DO NOT FREEZE.** Keep plate tightly closed at all times. Between uses, plates may be stored inverted in their mylar pouches. The expiration date is listed on the box label.
### Notes:

![Graph showing concentration of Ig mg/100ml against ring diameter (in mm).](image-url)
VMRD, Inc.’s radial immunodiffusion plates are ready-to-use, with standards calibrated to determine samples within the normal range. Custom plates can be prepared to meet your specific needs.

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**IMMUNOGLOBULIN DISORDERS IN DOGS**

*Hypergammaglobulinemia:* This term is used to describe a significant increase in circulating immunoglobulin levels. Based on serum electrophoresis, hypergammaglobulinemias are divided into monoclonal and polyclonal gammopathies.

*Monoclonal gammopathies* are recognized by the presence of a very sharp spike on the serum electrophoresis scan. This spike is caused by immunoglobulin-producing tumor cells, usually plasma cells (multiple myelomas). In the dog, these neoplastic plasma cells may secrete either IgG, IgM or IgA. Since the tumors arise from a single cell and secrete only one class of
immunoglobulin with only one variable region, a monoclonal spike is noted on electrophoretic evaluation. A monoclonal gammopathy detected by serum electrophoresis can be characterized and quantified by single radial immunodiffusion.

Myelomas in dogs are often recognized initially as bleeding problems. In addition, hyperviscosity occurs from the large amounts of immunoglobulin in the serum resulting in congestive heart failure, retinopathy, and neurologic signs. Hyperviscosity is most severe in the case of IgM myelomas (macroglobulinemia). Tumor cells result in masses in the bones leading to osteolytic and osteoporotic lesions.

**Polyclonal gammopathies** are characterized by an overall increase in immunoglobulins and are seen as a broad increase in the gamma region of an electrophoresis scan. The common causes of this type of hypergamma-globulinemia in the dog are chronic infections, neoplasms, and certain autoimmune disorders.

**Immunodeficiency Disorders:**
Although primary immunodeficiencies which affect immunoglobulin production are rare in dogs, the evaluation of serum immunoglobulin levels in dogs with persistent infections is recommended. A major reason is that secondary immunodeficiencies are associated with infections, neoplasia, failure to obtain colostrum, and other conditions.
## IMMUNOGLOBULIN CONCENTRATIONS

(mg/100 ml)

<table>
<thead>
<tr>
<th>Puppy</th>
<th>Age</th>
<th>Ref.</th>
<th>IgA</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2 wks.</td>
<td>7</td>
<td>Undetectable</td>
<td>56 (36-70)</td>
<td>73 (60-100)</td>
</tr>
<tr>
<td></td>
<td>2 mos.</td>
<td>7</td>
<td>Undetectable</td>
<td>79 (50-170)</td>
<td>99 (68-130)</td>
</tr>
<tr>
<td>Grey Collie</td>
<td>2 mos.</td>
<td>7</td>
<td>30 (0-46)</td>
<td>206 (100-440)</td>
<td>136 (70-250)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Normal Adult Dog Sera</th>
<th>No. of Dogs</th>
<th>Ref.</th>
<th>IgA</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mongrel</td>
<td>20-35</td>
<td>3</td>
<td>79 (30-240)</td>
<td>1445</td>
<td>145 ± 65</td>
</tr>
<tr>
<td>Foxhound &amp; Collie</td>
<td>21-37</td>
<td>3</td>
<td>83 (40-240)</td>
<td>925</td>
<td>156 (70-300)</td>
</tr>
<tr>
<td>Mongrel</td>
<td>14</td>
<td>4</td>
<td>50 (20-120)</td>
<td>980 (520-1730)</td>
<td>170 ± 100</td>
</tr>
<tr>
<td>Purebred &amp; Mongrel (pooled samples)</td>
<td>1000</td>
<td>5</td>
<td>100 ± 60</td>
<td>1500 ± 500</td>
<td>150 ± 50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Normal Colostrum</th>
<th>No. of Dogs</th>
<th>Ref.</th>
<th>IgA</th>
<th>IgG</th>
<th>IgM</th>
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<tbody>
<tr>
<td>Mongrel</td>
<td>7-12</td>
<td>-</td>
<td>313 (170-520)</td>
<td>1453</td>
<td>217 (70-370)</td>
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</table>

<table>
<thead>
<tr>
<th>Milk</th>
<th>No. of Dogs</th>
<th>Ref.</th>
<th>IgA</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>-</td>
<td>8</td>
<td>110-620</td>
<td>1-3</td>
<td>10-54</td>
</tr>
</tbody>
</table>
References


