OF THE many methods described for detecting microfilariae in the blood, the direct smear, the Knott’s test and the filter technique appear to be most widely recommended and used.

The filter technique, as originally described, uses a lysing solution containing methylene blue (1:10,000). After this solution has been mixed with blood, the hemolyzed specimen is injected through a 25-mm., 8-micron Millipore filter pad which traps the microfilariae.

Several technical difficulties have been encountered with this filter technique. The most common problem is that some samples (approximately 20%) fail to pass through the filters, either in part or completely. This problem has been attributed to fragmented cell ghosts, nuclear debris and mucoid ground substance of cytoplasmic origin which readily clog the pores of the opaque membranes. Other problems, including overstaining of the filter membrane, poor staining of microfilariae, excessively stained cellular debris, rapid drying of the filter membrane and excess depth of field, make identification and differentiation of microfilariae difficult.

A commercially available test kit, the Difil Test (EVSCO Pharmaceutical Corp.), utilizing the principle of the filtration technique, overcomes many of these problems.

In the Difil Test method, 1 ml. of blood is drawn directly from the dog and immediately diluted and lysed with the Difil Test lysing solution, which contains an anticoagulant. Alternately, if performance of the test is to be deferred, blood can be drawn into any anticoagulant such as EDTA, heparin or sodium citrate. The syringe is firmly attached to the lysing-solution dispenser bottle (Figure 1) and approximately 8 ml. to 10 ml. of the lysing solution is aspirated. The amount of
The gentle helping hand for many procedures.

Just reach for ROMPUN®

sedative/analgesic for dogs and cats

ROMPUN is truly unique in the field of veterinary medicine. Exceptional versatility is a hallmark of ROMPUN sedative/analgesic, but not its sole virtue. ROMPUN is non-narcotic. It produces a pain-easing sedation which resembles natural sleep, yet is interruptable by external stimuli. A wide safety margin and minimal side effects also characterize this product of many uses. Its high standard of dependability has been verified by numerous practicing veterinarians. The exceptional versatility provides you with an opportunity to use ROMPUN to facilitate a multitude of procedures in your practice. Use it with confidence.

Potent sedation and analgesia. Sedation can be maintained for 1 to 2 hours, depending on the dosage. Analgesia lasts from 15 to 30 minutes, and is far superior to the analgesic effect of tranquillizers. As a pre-anesthetic agent, ROMPUN can reduce the requirement of barbiturates from ¼ to ½ of the calculated dosage to produce a surgical plane of anesthesia.

Soothes...calms...relaxes. ROMPUN acts immediately to relieve discomfort of injured or post-operative animals. And to calm frightened or fractious patients. A centrally-acting muscle relaxant, ROMPUN induces relaxation of skeletal musculature.

IV-IM-SC. Use ROMPUN intravenously, intramuscularly, subcutaneously, for restraint, minor procedures or preanesthesia.

An intramuscular or subcutaneous dosage (1 mg. per pound of body weight) produces the maximum effect within 10 to 15 minutes; intravenous dosage (0.5 mg.) within 3 to 5 minutes.

Fast, predictable recovery. Patient emerges as if waking from a deep sleep usually within 1 to 2 hours after injection. Post-anesthetic or emergence excitement has not been observed in animals preanesthetized with ROMPUN.

Order now from your wholesale Animal Health Department, Chemag Division of Baychem Corporation, Bc 2037, Shawnee Mission, Kansas 6620
Figure 1
Approximately 1 ml. of blood is drawn into a 10-ml syringe and 8 ml. to 9 ml. of lysing solution is aspirated.

Figure 2
The hemolysed specimen is agitated to lyse both red and white cells.

Figure 3
The clear, colorless filter membrane is loaded onto the gauze mesh of the filter holder and the cap is secured.
Figure 4 (above)—The lysed cell solution is injected slowly through the filter holder.

Figure 5 (above right)—One or two flushes with water are used to remove excess cellular debris from the filter.

DEFIL TEST KIT (CONT'D)

Air drawn into the syringe allows for mixing. The syringe is agitated four or five times to allow for complete lysis of both red and white cells (Figure 2). The lysing solution does not destroy the microfilariae.

A specially designed clear filter membrane is loaded onto the gauze mesh of the filter holder (Figure 3) and the cap is firmly secured. With the syringe connected to the filter holder, the lysed cell solution is injected slowly through the system (Figure 4). This procedure is followed by one or two flushings with 8 ml. to 10 ml. of water to clear the membrane of excess debris (Figure 5). To avoid flushing trapped microfilariae through the membrane, this step

Figure 6
The undersurface of the filter holder is blotted to drain water from around the membrane. The cap is unscrewed and the filter is removed, as in Figure 3, and transferred to a glass slide.
must be performed slowly.

The undersurface of the filter holder is blotted to drain excess water from around the filter (Figure 6). The cap is removed and the filter gently transferred with thumb forceps to a clean glass slide, keeping the filtered material uppermost. Staining is carried out using 1 or 2 drops of Difil Test stain (Figure 7). A coverslip is placed over the stained filter and excess stain is removed by pressing the coverslip to the slide between a gauze pad or paper towel. The preparation is then ready for microscopic examination (Figure 8).

The slide can be rapidly scanned under low-power magnification (10X). When the Difil Test technique is used, microfilariae stain reddish purple (Figure 9). They may be differentiated by morphology and measurement of length. The filter holes, which are visible microscopically, are 8 microns in diameter. This measurement can be used as a guide in determining the width of microfilariae at the nerve ring.
The length of microfilariae of Dirofilaria immitis and Dipetylonema reconditum appears shorter with this method than previously described.\textsuperscript{5,6} Table 1 lists the differential features used to distinguish the two species of canine filariae when this method is used. Length, width and such anatomic features as the tapered or blunt head, presence or absence of a cephalic hook\textsuperscript{5} or button-hook tail, are the major features by which microfilariae may be differentiated.

**Comparison of the Filter Technique to Other Methods**

Several authors have published data comparing the other common methods of microfilariae detection (Table 2) to the filter technique.

In most cases, the filtration technique appears to be more accurate in detecting microfilariae, especially when the numbers of parasites in the circulating blood are few.\textsuperscript{12} This is true except in reported instances\textsuperscript{5,6} when the technical difficulties previously mentioned led the authors to abandon this method.

Using blood samples from 846 dogs from the New York City metropolitan area, our laboratory compared the direct wet smear technique to the modified filtration test. Of the 846 samples, 27 (3.2\%) were positive by the direct smear, while 34 (4.0\%) were positive using the Difil Test. The Difil Test detected 21\% more dogs with heartworm disease than did the conventional method.

The absence of circulating microfilariae in the blood does not necessarily exclude the possibility of heartworm infestation. In approximately 5\% to 10\% of the dogs with *D. immitis* infestation, microfilariae cannot be detected in the circulating blood.\textsuperscript{13} Inadequate sample size, immaturity, sterility, monogamy, host immunity or prior therapy are some of the reasons for these false negative results.

When heartworm infestation is suspect-

**TABLE 1—Canine Microfilariae Identification Using the Filter Technique**

<table>
<thead>
<tr>
<th></th>
<th><em>D. reconditum</em></th>
<th><em>D. immitis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Average length (microns)</td>
<td>213-240</td>
<td>240-273</td>
</tr>
<tr>
<td>Width (microns) at nerve ring</td>
<td>4.7-5.8</td>
<td>6.1-7.2</td>
</tr>
<tr>
<td>Anterior extremity</td>
<td>Narrower than filter holes</td>
<td>As wide as the filter holes</td>
</tr>
<tr>
<td>Tail</td>
<td>Button-hooked</td>
<td>Straight</td>
</tr>
<tr>
<td>Number</td>
<td>Few</td>
<td>Many</td>
</tr>
<tr>
<td>Cephalic hook</td>
<td>Present</td>
<td>Absent</td>
</tr>
</tbody>
</table>

**TABLE 2—Comparison of Filter Technique to Other Methods for Detecting Canine Microfilariae**

<table>
<thead>
<tr>
<th>Author</th>
<th>Geographic Region</th>
<th>Rel.</th>
<th>No. of Dogs Tested</th>
<th>% Positive for Microfilariae (Filtration Method)</th>
<th>Positive for the Filtration Technique but Negative for:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altman, N. H.</td>
<td>Baltimore</td>
<td>2</td>
<td>1800</td>
<td>26.7% ND 5.0% 1.1%</td>
<td>D. reconditum Capillary Knott Method</td>
</tr>
<tr>
<td>Wylie, J. P.</td>
<td>Boston</td>
<td>6</td>
<td>25</td>
<td>100% 17% 18% 4%</td>
<td></td>
</tr>
<tr>
<td>Wylie, J. P.</td>
<td>Boston</td>
<td>6</td>
<td>202</td>
<td>25% ND ND 1.9%</td>
<td></td>
</tr>
<tr>
<td>Palumbo, N. E.</td>
<td>Hawaii</td>
<td>7</td>
<td>85</td>
<td>23.5% 1.2% ND</td>
<td></td>
</tr>
<tr>
<td>Watson, A. D. J.</td>
<td>Sydney, Australia</td>
<td>11</td>
<td>495</td>
<td>8.1% ND ND 17.5%</td>
<td></td>
</tr>
<tr>
<td>Wilkins, R. J.</td>
<td>New York</td>
<td>*</td>
<td>846</td>
<td>4.0% 21% ND ND</td>
<td></td>
</tr>
</tbody>
</table>

ND - Not Done  *Present study
ed clinically we have found collection of blood samples in the evening to be beneficial. Other diagnostic tests used to confirm a diagnosis include thoracic radiographs, electrocardiogram, routine blood counts, total eosinophil counts and serum protein electrophoresis.

Conclusion
The Difil Test kit appears to be a reliable working application of the filtration technique for the detection and differentiation of circulating microfilariae in the blood.

The technical improvements and modification in lysing solution, staining technique and use of a clear transparent filter membrane have minimized residual cellular debris and eliminated many of the problems inherent in the original method.

Because the filters effectively trap all microfilariae in the blood sample, quantitation of microfilariae recovered is possible, providing a known blood volume is used.

In addition to its reliability, the filter technique is very rapid; no centrifugation is involved and little time is required to scan the slide. This method should allow practitioners to screen blood samples for circulating microfilariae quickly and accurately.

ACKNOWLEDGMENTS
The technical assistance of Miss Denise Vito and Mr. Normal Greenbury is acknowledged.

REFERENCES