Comparison of 2 doses of recombinant human thyrotropin for thyroid function testing in healthy and suspected hypothyroid dogs.

Boretti, F S; Sieber-Ruckstuhl, N S; Wenger-Riggenbach, B; Gerber, B; Lutz, H; Hofmann-Lehmann, R; Reusch, C E
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Abstract

BACKGROUND: Various protocols using different doses of recombinant human thyrotropin (rhTSH) in TSH stimulation testing have been described. However, the influence of TSH dosage on thyroxine (T4) concentration has not yet been evaluated in suspected hypothyroid dogs. OBJECTIVE: To evaluate the effectiveness of 2 doses of rhTSH. ANIMALS: Fifteen dogs with clinical signs consistent with hypothyroidism and abnormal stimulation results with 75 microg rhTSH and 18 clinically healthy dogs. METHODS: All dogs were stimulated with 75 and 150 microg rhTSH IV in a 1st and 2nd stimulation test, respectively. Blood samples were taken before and 6 hours after rhTSH administration for determination of total T4 concentration. RESULTS: Using the higher dose led to a normal test interpretation in 9 of the 15 dogs, in which stimulation had been abnormal using the lower dose. Based on follow-up information, hypothyroidism was excluded in 7 of these 9 dogs. In all 6 dogs with a blunted response to the higher dose, hypothyroidism could be confirmed. Healthy dogs showed significantly higher post-TSH T4 concentrations with the higher compared with the lower dose. Post-TSH T4 concentrations after TSH stimulation were not related to dogs' body weight in either healthy or diseased dogs. CONCLUSIONS AND CLINICAL RELEVANCE: TSH dose significantly influenced test interpretation in suspected hypothyroid dogs. Differentiation between primary hypothyroidism and nonthyroidal disease was improved with 150 microg rhTSH. Because this effect was independent of the dogs' body weight, the higher dose is recommended in dogs that have concurrent disease or are receiving medication.
Comparison of 2 Doses of Recombinant Human Thyrotropin for Thyroid Function Testing in Healthy and Suspected Hypothyroid Dogs


Background: Various protocols using different doses of recombinant human thyrotropin (rhTSH) in TSH stimulation testing have been described. However, the influence of TSH dosage on thyroxine (T4) concentration has not yet been evaluated in suspected hypothyroid dogs.

Objective: To evaluate the effectiveness of 2 doses of rhTSH.

Animals: Fifteen dogs with clinical signs consistent with hypothyroidism and abnormal stimulation results with 75 μg rhTSH and 18 clinically healthy dogs.

Methods: All dogs were stimulated with 75 and 150 μg rhTSH IV in a 1st and 2nd stimulation test, respectively. Blood samples were taken before and 6 hours after rhTSH administration for determination of total T4 concentration.

Results: Using the higher dose led to a normal test interpretation in 9 of the 15 dogs, in which stimulation had been abnormal using the lower dose. Based on follow-up information, hypothyroidism was excluded in 7 of these 9 dogs. In all 6 dogs with a blunted response to the higher dose, hypothyroidism could be confirmed. Healthy dogs showed significantly higher post-TSH T4 concentrations with the higher compared with the lower dose. Post-TSH T4 concentrations after TSH stimulation were not related to dogs’ body weight in either healthy or diseased dogs.

Conclusions and Clinical Relevance: TSH dose significantly influenced test interpretation in suspected hypothyroid dogs. Differentiation between primary hypothyroidism and nonthyroidal disease was improved with 150 μg rhTSH. Because this effect was independent of the dogs’ body weight, the higher dose is recommended in dogs that have concurrent disease or are receiving medication.

Key words: Diagnosis; Hypothyroidism; rhTSH; Stimulation.

Although hypothyroidism is one of the most common endocrinopathies in dogs, its diagnosis still can be challenging. Determination of serum thyroxine concentration (T4) is accepted as a primary screening test because of its good diagnostic sensitivity, but its specificity is low because it is influenced not only by non-thyroidal disease but also by many drugs. In contrast to the situation in human medicine, the low sensitivity of endogenous thyrotropin (TSH) so far has precluded its use as a reliable diagnostic test in veterinary medicine.1-3 The TSH stimulation test has long been considered an accurate test, and some authors still recognize it as the “gold standard” for confirming the diagnosis of canine hypothyroidism.4 Determination of circulating T4 before and after administration of exogenous TSH provides an assessment of the functional reserve capacity of the thyroid gland, with minimal to no stimulation expected in hypothyroidism. The test seems to be less influenced by non-thyroidal illness and medications known to affect thyroid function.4 Because the traditionally used bovine TSH (bTSH) is no longer commercially available as a pharmaceutical preparation, a number of protocols with recombinant human TSH (rhTSH) have been proposed for TSH stimulation testing in dogs.5-8 In the 1st study evaluating rhTSH in healthy Beagles, the authors proposed a dose of 50 μg rhTSH per dog.8 Additional studies used higher doses (75 and 91 μg)9,10 or doses based on the dogs’ body weight, with 50 μg for dogs below 29 kg and 100 μg rhTSH for dogs above 29 kg.10 Because the cost of rhTSH is high, using a lower dose would be helpful in decreasing the overall expense of TSH stimulation testing and make the test useful for veterinarians in private practice. On the other hand, using a lower dose could lead to less than maximal stimulation in a dog with non-thyroidal disease, which in turn could result in misdiagnosis and unnecessary treatment of a euthyroid dog. In situations in which testing cannot be delayed or in which medication cannot be withdrawn, knowledge of the optimal dose to clearly differentiate between primary hypothyroidism and non-thyroidal disease would be a prerequisite for performing the test. However, the influence of rhTSH dose so far has not been systematically evaluated in diseased dogs.

Consequently, the purpose of the present study was to evaluate the effectiveness of 2 doses of rhTSH to assess thyroid function in dogs, in which hypothyroidism was suspected. Therefore, dogs in which stimulation...
with 75\(\mu\)g rhTSH had been considered abnormal were restimulated a 2nd time with 150\(\mu\)g rhTSH. In addition, healthy, body weight-matched control dogs were stimulated with both doses to assess a possible influence of body weight on magnitude of stimulation.

**Materials and Methods**

**Dogs Suspected of Having Hypothyroidism**

Dogs that had been presented or referred to the Clinic for Small Animal Internal Medicine of the University of Zurich for investigation of clinical signs consistent with hypothyroidism underwent a 1st TSH stimulation test (1st stimulation). Results of stimulation tests were interpreted with criteria established in 38 healthy dogs. On the basis of these criteria, dogs were assigned to 1 of 3 groups, depending on whether they showed normal, no, or intermediate stimulation. A normal stimulation was defined as a post-TSH T4 of \(\geq 2.5\)\(\mu\)g/dL and at least 1.5 times the basal T4 concentration. Dogs with a post-TSH T4 of \(<1.6\)\(\mu\)g/dL and \(<1.5\) times the basal T4 concentration were considered to have no stimulation. Dogs with a post-TSH T4 between 1.6 and 2.5 or post-TSH T4 values of \(\geq 2.5\)\(\mu\)g/dL, but an increase of \(<1.5\) times basal T4 was considered to have an intermediate stimulation.

Dogs were included in the present study if the 1st stimulation was considered abnormal (no or intermediate stimulation) and if owners agreed to a 2nd stimulation. A total of 15 dogs with a median (range) age of 6 years (2–12 years) and a median (range) body weight of 32 kg (18–104 kg) fulfilled the inclusion criteria. The most common complaints, either alone or in combination, were exercise intolerance, dermatologic problems, and weight gain. Medication history included, either alone or in combination, glucocorticoids (prednisolone, 0.2–1.5 mg/kg q12h for 8–12 weeks; hydrocortisone 10\(\)mg/dL; each concentration tested in duplicate twice daily over the course of 20 days) were 3.9–10.8 and 5.2–13.8%, respectively (reference range, 1.5–5.0\(\mu\)g/dL). Serum cTSH concentrations were determined in prestimulation samples on the day of the 1st TSH stimulation by use of a solid part, 2-site chemiluminescent enzyme immunometric assay validated for use in dogs (upper limit of the reference range, 0.6 ng/mL).

**Analytical Procedures**

Serum T4 was measured by use of a commercially available solid phase, chemiluminescent enzyme immunoassay validated for use in dogs; intraassay and interassay coefficients of variation (6 T4 concentrations, 0.65–11.9\(\mu\)g/dL; each concentration tested in duplicate twice daily over the course of 20 days) were 1.5–2.5%.

**Statistical Analyses**

Data were analyzed by nonparametric statistical methods. Wilcoxon’s matched pairs, Friedman’s repeated measures test for paired samples, and Dunn’s multiple comparisons test were used. The Mann-Whitney U-test was used to determine differences between the 2 groups. For continuous variables, linear correlation and the nonparametric Spearman rank test were calculated. Values of \(P < .05\) were considered statistically significant.

**Results**

No adverse reactions were noticed in any of the dogs after IV administration of repeated doses of rhTSH.

**Stimulation Results in Clinically Healthy Dogs**

Median (range) T4 and post-TSH T4 concentrations with 75 and 150\(\mu\)g are shown in Table 1. Calculation of 5 and 95% quartiles with 75 and 150\(\mu\)g rhTSH yielded post-TSH T4 concentrations of 2.0 and 5.5\(\mu\)g/dL, and 2.2 and 5.9\(\mu\)g/dL, respectively. All dogs showed a significant increase (\(P = .0002\)) in T4 after administration of 75 and 150\(\mu\)g rhTSH, respectively. However, using 150\(\mu\)g rhTSH led to a significantly higher post-TSH T4 concentration (\(P = .003\)) compared with the lower dose (Fig 1A). With the exception of 1 dog, all participating dogs met the stimulation criteria for euthyroidism with both dosages. The one with an intermediate result had post-stimulation T4 concentrations of 2.0 and 5.9\(\mu\)g/dL with 75 and 150\(\mu\)g rhTSH, respectively.

There was no significant correlation between post-TSH T4 concentrations and the dogs’ body weight.

**Table 1.** T4 and post-TSH T4 concentrations (median and range) in 18 healthy dogs stimulated with 75 and 150\(\mu\)g rhTSH.

<table>
<thead>
<tr>
<th>Variable</th>
<th>75(\mu)g rhTSH</th>
<th>150(\mu)g rhTSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4 ((\mu)g/dL)</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>Post TSH T4 ((\mu)g/dL)</td>
<td>3.9–5.5</td>
<td>4.5–5.9</td>
</tr>
</tbody>
</table>

T4, thyroxine; rhTSH, recombinant human thyrotropin.
Stimulation and cTSH Results of Dogs Suspected to Have Hypothyroidism

Median (range) T4 and post-TSH T4 concentrations with 75 and 150 µg are presented in Table 2; they were significantly lower compared with the healthy control dogs (P < .001). There was no significant difference in post-TSH T4 concentration with the higher compared with the lower dose (Fig 1B; P = .146). However, using 150 µg rhTSH led to a normal test interpretation in 9 of the 15 dogs according to criteria established in healthy dogs with 75 µg rhTSH, whereas in the other 6, stimulation still was blunted (2 dogs with intermediate response, 4 dogs with no response). Post-TSH T4 concentrations and increases in Post-TSH T4 concentrations of the 6 and 9 dogs using both doses are presented in Table 3 and Table 4, respectively.

Median (range) body weight of the 6 and 9 dogs was 37 kg (31–76 kg) and 30 kg (18–104 kg), respectively. There was no significant correlation between post-TSH

T4 concentrations and the dogs’ body weight (r = −0.19; P = .5). Four of the 9 dogs in which stimulation was normal using the higher dose had body weights below 30 kg, and one of them had a body weight below 20 kg.

Median (range) cTSH concentrations in the 15 dogs were 0.35 ng/mL (0.05–2.2 ng/mL), with 2 dogs having cTSH concentrations above the reference range. Excluding 4 dogs from statistical analysis, in which the 2nd stimulation had been performed more than 2 weeks after the 1st stimulation did not lead to significantly higher post-TSH T4 concentrations using the higher compared with the lower dose (P = .496).

Follow-Up Information on the Dogs, in Which the 2nd Stimulation using the Higher Dose Was Still Abnormal (n = 6)

Hypothyroidism could be confirmed in all 6 dogs with a blunted response to the 2nd stimulation based on concurrent laboratory findings (anemia, hypercholesterolemia, hypertriglyceridemia, increased cTSH concentration) and follow-up information (3rd TSH-stimulation test, improvement or resolution of clinical signs after 4 weeks of thyroxine supplementation).

Follow-Up Information on the Dogs, in Which the 2nd Stimulation using the Higher Dose Was Normal (n = 9)

In 5 of the 9 dogs with a normal stimulation using the higher dose, the owners agreed to a 3rd stimulation using the

Table 2. T4 and post-TSH T4 concentrations (median and range) in 15 dogs suspected of having hypothyroidism stimulated with 75 and 150 µg rhTSH.

<table>
<thead>
<tr>
<th>Variable</th>
<th>75 µg rhTSH</th>
<th>150 µg rhTSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4 (µg/dL)</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>0.7–3.5</td>
</tr>
<tr>
<td>Post-TSH T4</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>(µg/dL)</td>
<td>2.0</td>
<td>1.1–4.7</td>
</tr>
</tbody>
</table>

T4, thyroxine; rhTSH, recombinant human thyrotropin.
the lower dose, which was performed after a median (range) of 19 (12–24) months after the 1st stimulation. Post-TSH T4 concentrations were normal in 3 of them and they were clinically unremarkable with normal laboratory test results. Therefore, hypothyroidism was excluded. Two of the 3 had received NSAIDS during the time when the 1st 2 stimulation tests had been performed; the other had been diagnosed with hypoadrenocorticism and had received 0.5 mg/kg prednisolone q24h in addition to fludrocortisone. When the 3rd stimulation was performed, NSAIDS and prednisolone had been withdrawn.

Two of the 5 dogs had a post-TSH T4 concentration of 1.7 μg/dL after the 3rd stimulation with a basal T4 concentration of 0.5 μg/dL (cTSH, 0.27 ng/mL) and 1.3 μg/dL (cTSH, 0.7 ng/mL), respectively. Nineteen and 23 months previously, post-TSH T4 concentrations using the higher rhTSH dose had been 2.6 and 3.0 μg/dL, respectively. Both dogs still had recurrent dermatologic problems and had been treated intermittently with antibiotics and glucocorticoids not only during the 1st 2 but also at the time of the 3rd stimulation. Routine laboratory test results were within normal limits, and the owners decided against levothyroxine supplementation at that time. Therefore, hypothyroidism could neither be clearly confirmed nor excluded.

In 4 of the 9 dogs, follow-up information only was available by telephone contact with the owner or the veterinarian. All 4 dogs had been treated with antibiotics, glucocorticoids, or both at the time the TSH stimulation tests had been performed. Another diagnosis had been made, and the dogs had been treated accordingly. The owners reported that the dogs were doing well with no clinical signs and without medication 12 (2 dogs), 21, and 24 months after the 1st stimulation, respectively. Hypothyroidism therefore was considered unlikely.

Overall, based on our follow-up information, hypothyroidism is highly unlikely at least in 7 of the 9 dogs with a normal 2nd stimulation. In the other 2 dogs, hypothyroidism could neither be clearly confirmed nor excluded. Differentiation between primary hypothyroidism and nonthyroidal disease therefore was improved by using the higher rather than the lower dose.

### Discussion

The purpose of the present study was to evaluate the effectiveness of 2 doses of rhTSH in assessing thyroid function in healthy and suspected hypothyroid dogs. We showed that in healthy dogs the magnitude of stimulation was significantly influenced by the rhTSH dosage, inasmuch as they had significantly higher post-TSH T4 concentrations using the higher rather than the lower dose. Although this observation could not be confirmed in the suspected hypothyroid dogs, using the higher dose led to a normal test interpretation in the majority of dogs. The lower dose therefore had resulted in a less than maximal stimulation in patients without hypothyroidism and could possibly have led to an inappropriate diagnosis of hypothyroidism if the 2nd stimulation had not been performed.

Most dogs were restimulated within 9 days. However, in 3 dogs several months had passed between the 1st and 2nd stimulation. In these dogs, a clinical change in the patient and the withdrawal of the medication may have influenced the test result of the 2nd stimulation. It is highly likely that even the lower dose would have led to a normal stimulation at that time point. An argument further supporting this assumption is the fact that those dogs with no clinical signs and not receiving medication had normal post-TSH T4 concentrations at the 3rd stimulation using the lower dose. The TSH-stimulation test also can be blunted in dogs with nonthyroidal disease or in dogs treated with glucocorticoids.4,11 However, the practicing veterinarian can be presented with dogs suspected of having hypothyroidism that are already being treated with medication at the time of testing or have a concurrent disease. As indicated by our results, the suppressive effect of drugs or nonthyroidal illness seems to be less pronounced using a higher dose of TSH. Therefore, to improve the discriminatory ability of the TSH stimulation test to differentiate between nonthyroidal illness and primary hypothyroidism, the higher dose should be recommended in dogs, in which testing cannot be delayed.

Interestingly, the responsiveness of the dogs to the stimulations with 2 different doses was not body weight dependent. Although in our healthy dogs, post-TSH T4 concentrations were significantly higher with the higher dose, this difference also was unrelated to the dogs’ body weight. This finding clearly is an argument against a weight-related phenomenon. Therefore, a dosage recommendation based on a body weight category (ie, 50 and

### Table 3. Post-TSH T4 concentrations (median, range) at both dosages in 9 and 6 dogs in which stimulation using the higher dose was considered normal and still abnormal, respectively.

<table>
<thead>
<tr>
<th>Post-TSH T4 (μg/dL)</th>
<th>Normal 2nd stimulation (9 dogs)</th>
<th>Abnormal 2nd stimulation (6 dogs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>75 μg rhTSH</td>
<td>2.2</td>
<td>(1.8–4.7)</td>
</tr>
<tr>
<td>150 μg rhTSH</td>
<td>2.7</td>
<td>(2.6–3.5)</td>
</tr>
</tbody>
</table>

T4, thyroxine; rhTSH, recombinant human thyrotropin.

### Table 4. X-fold increase (median, range) of post-TSH T4 concentrations at both dosages in 9 and 6 dogs in which stimulation using the higher dose was considered normal and still abnormal, respectively.

<table>
<thead>
<tr>
<th>X-fold Increase of Post-TSH T4</th>
<th>Abnormal 2nd Stimulation (9 dogs)</th>
<th>Normal 2nd Stimulation (6 dogs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>75 μg rhTSH</td>
<td>1.5</td>
<td>(1.3–2.4)</td>
</tr>
<tr>
<td>150 μg rhTSH</td>
<td>2.3</td>
<td>(1.5–2.9)</td>
</tr>
</tbody>
</table>

T4, thyroxine; rhTSH, recombinant human thyrotropin.
100 µg rhTSH for body weight below and above 30 kg, respectively), as has been suggested,\(^6\) does not seem to be reasonable. Alternatively, we had 5 suspected hypothyroid dogs with body weights below 30 kg, in which only stimulation with the higher dose was considered normal.

A dose- but not weight-related increase in post-TSH T4 concentration was already demonstrated in the 1st study evaluating rhTSH.\(^8\) The authors compared stimulation results using 50 and 100 µg rhTSH in a group of healthy Beagle dogs of similar body weight. Although not statistically significant, the higher dose led to higher post-TSH T4 concentrations. The results of that study, like ours, indicate that using a higher dose leads to a higher stimulation, a fact providing evidence that the maximal stimulation of the thyroid gland has not yet been reached. Use of a low dose of rhTSH might therefore result in less than maximal stimulation and hence lead to a different test interpretation. Although our results show that the higher dose of rhTSH resulted in a greater T4 response especially in dogs, in which thyroid gland was suppressed by concurrent disease or by medication, no conclusions on the optimal dose of rhTSH can be drawn as an actual dose response study was not performed.

Therefore, the question arises whether criteria for test interpretation must be adjusted or modified for each dosage, as has been proposed in an earlier study using the bovine TSH preparation.\(^12\) The authors evaluated 2 doses of bTSH in healthy dogs and found a significantly lower response to the low dose of bTSH (1 IU) than to the high dose (5 IU) of bTSH. Notably, this effect also was observed to be independent of the dogs’ body weights. The authors concluded that the lower dose can be used to perform the test, but they recommended that criteria for test interpretation should be modified from those that are used for the higher dose. Various criteria for test interpretation can be found in the literature on using rhTSH.\(^6–9,13\) In all but 1 of those studies, criteria merely have been adapted from earlier experience with the bovine TSH preparation. Although the bioactivity per microgram of TSH of the recombinant human preparation seems to be higher,\(^14\) compared with that of the bovine preparation, one could assume that using 75 µg rhTSH must be considered a low-dose TSH stimulation test, as suggested by Beale and colleagues for the 1 IU bTSH dose. A recent study\(^13\) suggested that criteria for test interpretation using rhTSH that were proposed in early and experimental studies might be too stringent. The authors therefore defined new criteria based on their own experience but not on systematic testing.

In a previous study, we evaluated rhTSH in 38 healthy dogs of different breeds\(^6\) and calculated reference ranges for post-TSH T4 concentration as 5, 50, and 95% quantiles. The 5% quantile post-TSH T4 concentration in that study was 2.5 µg/dL. This value is quite different from the 5% quantile calculated in the present study of 2.0 and 2.2 µg/dL using 75 and 150 µg rhTSH, respectively. Comparing the ages of the 38 dogs in the earlier study with those of the 18 healthy dogs in the present study revealed that the former were significantly younger (\(P = .001\); data not shown). Age-related changes in the secretory capacity of the thyroid gland might be responsible for this observation, as has been shown for the unstimulated, physiological T4 concentration.\(^15,16\) Reference values in general, or in our case criteria for test interpretation, should best be established not only in a healthy population but also in a population with an age range in which the disease is expected to occur. Recalculating the 5% quantile including all healthy dogs (38 of the previous study including 18 purpose bred dogs and 18 of the present study) resulted in a post-TSH T4 concentration of 2.2 µg/dL. Excluding the 18 Beagle dogs results in an even lower post-TSH T4 concentration of 2.0 µg/dL. Using the low-dose rhTSH stimulation test with the 2.2 µg/dL post-TSH T4 concentration as the criterion for a normal stimulation leads to a difference in test interpretation compared with the higher dose in only 4 of the 15 dogs.

The main limitation of our study, but also of most others evaluating thyroid function, is the lack of a gold standard with which the true thyroid status of the dogs can be determined. Approximately 75% of the thyroid gland must be destroyed before thyroid hormone production is diminished.\(^14\) Pre- or subclinical hypothyroidism at least in some of our healthy but older animals cannot be definitely excluded. Evaluating rhTSH tests in these animals might lead to a less pronounced response in T4 concentration, which in turn could lead to less stringent criteria for a normal response. In the present study, dogs were not randomly assigned to receive either the low or the high dose, which is a bias that could have affected our results. However, randomization would only have been feasible in the healthy dogs.

At present, the use of rhTSH in suspected hypothyroid dogs still necessitates prudence and increased vigilance, especially in patients with concurrent disease or on medication. More well-controlled studies are needed to determine the optimal dosage and possibly also the optimal criteria for test interpretation in suspected hypothyroid dogs, including those with subclinical hypothyroidism. Future studies should use a gold standard other than the TSH stimulation test, as has been proposed recently.\(^17\)

In conclusion, our study demonstrated that using the higher dose of rhTSH resulted in a higher discriminatory power with regard to differentiating between primary hypothyroidism and nonthyroidal disease. The magnitude of stimulation was influenced by the rhTSH dose but not by the dogs’ body weight. We recommend the higher dose of rhTSH for performing a TSH stimulation test in a diseased animal or an animal on medication, especially if testing cannot be delayed.

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**Footnotes**

\(^a\) Thyrogen, Genzyme Genzyme Corporation, Suffolk, UK

\(^b\) Immulite 1000 Canine Total T4, Diagnostic Products Corporation—DPC, Los Angeles, CA

\(^c\) Immulite Canine TSH, Diagnostic Products Corporation—DPC
Acknowledgments

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