Exotic Pet PRACTICE

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FEATURE ARTICLE

Quality Assurance in the In-House Laboratory

Terry W. Campbell, DVM, PhD

There are many advantages for the exotic animal clinician in obtaining in-house comprehensive laboratory data rather than sending samples to a reference laboratory. One goal of in-house laboratory testing is to decrease the turnaround time of results without compromising the quality. Analysis time is greatly reduced if testing is done in-house in most cases, especially if the tests, such as biochemical testing, can be performed on whole blood. The rapid turnaround time of test results is especially important in emergency situations. Another advantage of in-house testing may be cost efficiency. Often in-house methods, especially biochemical testing, are more effective and less costly than the use of a reference laboratory.[1] Many of the modern instruments are capable of performing biochemical tests on small sample volumes, which is especially important to the exotic animal clinician whose patients are "blood volume challenged."[2] The quality of in-house hematology results of exotic animals, especially those with nucleated erythrocytes and thrombocytes, is often better than that obtained from samples transported to outside laboratories because the sample can be analyzed within an hour of blood collection. Of course, the quality of test results depends on technical skills, the maintenance of the laboratory equipment, and a quality assurance program.

Only a documentable quality assurance program can guarantee the quality of test results day after day.[3] The quality assurance program for laboratory testing should include calibration of the instrumentation used, uses of controls to determine the precision and repeatability of the assays, and permanent documentation of test results. Calibration of an instrument is accomplished by running one or more samples (calibrators) with a known value for the test of interest, and adjusting the instrument reading to match those values. Instruments calibrated at the factory may require a periodical electronic function check to prevent errors that may occur with unskilled operators. Instruments without factory calibration may require periodic calibration. Calibration may be required to compensate for electronic drifts, each new lot of reagent, and changes in light sources and filters of spectrophotometers. The validity of a calibration (as well as the accuracy of the pipettes and reagents used) can be confirmed by running control samples before the instrument is used on patient samples.

Control samples are solutions that mimic a patient sample and have predetermined ranges of expected values. These ranges usually approach the lower and upper limits of the decision values of the clinician. Control samples are used to determine the precision or the repeatability of an assay. Commercially prepared controls are available for multiple analyzers. Controls should be run after each calibration to ensure the validity of the calibration and the accuracy of the test results. A calibrator should never be used as a control on the instrument it was used to calibrate. Within-run precision is accomplished by repeating a test 10 times consecutively. A coefficient of variation (CV) is calculated by dividing the standard deviation by the mean. The smaller the CV, the more precise the method. A CV less than 5% is adequate for analytes under tight homeostatic control, such as electrolytes; a CV less than 10% is adequate for others, such as enzymes.[3] Repeating the test on the control sample each day of testing allows for the determination of day-to-day precision. Plotting these data provides you with the opportunity to detect trends, including instrument drifts and reagent deterioration, and sudden shifts, such as a change in reagent lot, pipetting errors, and the occlusion of reagent lines.

Information regarding the accuracy, precision, and linearity of an

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Quality Assurance in the In-House Laboratory

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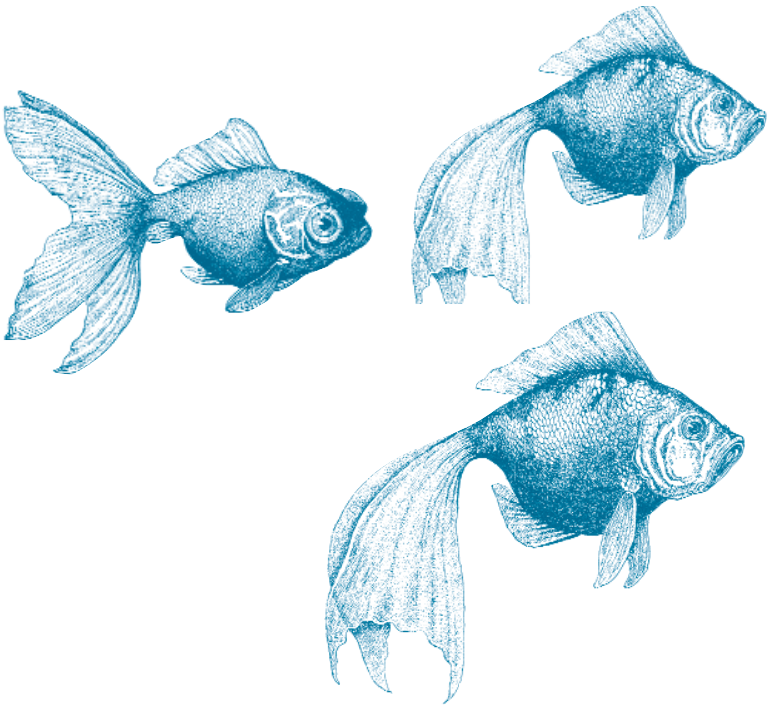
instrument is available from the company selling the instrument. *Accuracy* indicates how closely the measured value matches the true or expected value. *Precision* is the reproducibility of results for a given analyte. *Linearity* is the ability of an instrument to provide accurate measurements throughout a given range (usually the clinician's range of decision levels). The instrument should indicate when a concentration in the sample exceeds the linearity, thus allowing for testing of a diluted sample or use of another method to obtain a precise result. In such situations, reporting results greater than or less than a certain number may be satisfactory.

A variety of biochemical analyzers are available for in-house testing.[2] These can be discrete test analyzers, profile analyzers, or combination instruments. Discrete test analyzers are best used for single tests or small panels. They usually have the largest number of available tests and the greatest flexibility, but they are costly, in terms of both time and money, in running large panels. Profile analyzers require minimal technical skills to run and have a lower cost per test; however, single tests may be impossible or costly to run. Combination instruments may provide a compromise between cost and flexibility, but typically require more technical expertise to operate.

The management of the in-house laboratory and its quality assurance program should ideally be entrusted to one individual with the necessary level of interest, knowledge, and authority to train laboratory personnel. A well managed in-house clinical laboratory will provide rapid quality results because the instruments are well maintained, a quality assurance program is in place, and the proper technical training is provided.

References

1. Roth DF: Should you spring for in-house diagnostics? *Vet Econ* 33:58-64, 1992.
2. Vap LM, Mitzner B: An update on chemistry analyzers. *Vet Clin North Am Small Anim Pract* 26:1129-1154, 1996.
3. Vap LM: Point-of-care testing in the veterinary hospital. Presented at the 60th Annual Conference for Veterinarians. Fort Collins, Colo, January 9-11, 1999.



ROUNDTABLE

Alopecia in Rodents

Q. Which species are commonly affected by alopecia?

Dr Morrisey: *Any species of rodent can be affected by alopecia; I see it most in rats, mice, guinea pigs, and prairie dogs.*

Dr Worell: *I have commonly seen rats and mice presented with alopecia. Occasionally, a hamster, guinea pig, or chinchilla will be presented with the primary complaint of alopecia.*

Q. Discuss causes by species.

Dr Morrisey: *In rats and mice, the most common causes include barbering or self-mutilation. Ectoparasites such as mites and pinworms can also be a cause of alopecia. Secondary skin infection with bacteria or yeast can occur. In guinea pigs, alopecia may result from hormonal abnormalities, mange, ringworm, or folliculitis. I have also seen a number of prairie dogs with allergic dermatitis and secondary bacterial infection.*

Dr Worell: *Alopecia in chinchillas can come from a ringworm infection* (Trichophyton mentagrophytes) *or from fur-chewing by other chinchillas (aka, barbering) or by the chinchilla itself. Causes in guinea pigs include barbering, ectoparasites, vitamin deficiencies, and endocrine or hormonal changes. Alopecia in mice might be due to barbering, ectoparasites, or self-trauma from the environment. Causes for hamsters include barbering and ectoparasites, and alopecia may develop in rats because of barbering, ringworm, self-trauma, or ectoparasites.*

Q. Describe the clinical signs of alopecia.

Dr Morrisey: *Clinical signs, besides the obvious hair loss, include pruritus and excoriation of the skin. The hair loss may be bilaterally symmetrical in hormonal diseases. Flaky and scaly skin can occur with more chronic diseases.*

Dr Worell: *Clinical signs usually involve hair loss around the facial region and often the dorsal scapular area. Many of these affected animals are pruritic. Alopecia anywhere on the body is possible. Often there are scabs and crusts from intense pruritus. In cases of scabies in guinea pigs, there is often a thick, white, crusty material on the pet. Many of these affected guinea pigs will seizure when handled or when a skin scraping is performed.*

Q. Is the alopecia usually self-induced?

Dr Morrisey: *In some species it may be secondary to pruritus or a result of behavioral barbering.*

Dr Worell: *Depending on the situation, the alopecia may be self-induced due to pruritis or rubbing against objects in the environment.*

Q. What diagnostic tests are recommended?

Dr Morrisey: *A thorough history and complete physical examination are the first steps in diagnosing the problem. Skin scrape, cytology, and evaluation of the hair are also important. A skin biopsy with cultures can also be beneficial in determining the cause of the alopecia.*

Dr Worell: *Diagnostic tests include skin scrapings, Scotch tape preps, skin biopsies, and fungal cultures for dermatophytes.*

Q. What treatments do you recommend (including dosages)? Any topical therapies?

Dr Morrisey: *Treatment for barbering includes separating the affected animal from conspecifics. If the barbering is self-induced, then it can be difficult to control. Antihistamines (Atarax [Pfizer] 2 mg/kg PO tid), acepromazine in the drinking*

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WHAT'S YOUR DIAGNOSIS ???

Marine Aquarium in a Restaurant

Terry W. Campbell, DVM, PhD

You are called to examine a marine aquarium in a local restaurant. The aquarium is home to a variety of tangs (Acanthurus species). The owner of the aquarium complains that the fish seem to be doing a lot of rubbing on the rocks and dead coral in the exhibit. The owner informs you that all was fine with the aquarium until recently. He added two new tangs to the aquarium about 3 weeks ago. On close inspection, you notice that the fish are restless (darting suddenly), and a few are indeed rubbing on the rocks and bottom of the aquarium. They also exhibit increased gilling (respiratory effort), and you notice several whitish-gray nodules or spots on the skin of some of the fish. A water sample from the aquarium indicates normal water quality parameters.

Questions

1. What is the most likely diagnosis?
2. What other diagnostic tests would you perform?
3. What would be your recommended treatment?

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Alopecia in Rodents

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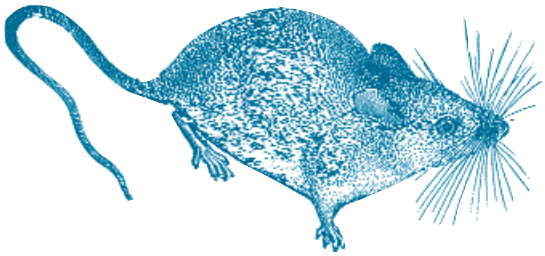

water (1 mg per 1 L water), or bandaging may be required to break the self-mutilation cycle. Appropriate antibiotics or antifungals are necessary if there is primary or secondary dermatitis. Ivermectin can be given orally or subcutaneously at 0.3 mg/kg or can be placed topically between the shoulder blades in mice at a dose of 2 mg/kg. In guinea pigs, ovarian cysts can cause the bilaterally symmetrical alopecia that responds to human chorionic gonadotropin (1000 IU IM) and ovariohysterectomy later. When using topical treatments, it's important to consider that the patient may ingest the material, therefore antibiotics that can cause dysbiosis (macrolides and penicillins) should not be used topically. Ivermectin should be given subcutaneously to guinea pigs and most other rodents at a dose of 0.3 mg/kg and repeated in 2 weeks. Also, topical and systemic steroids can cause anorexia, dysbiosis, and death and should not be used or should be used with extreme caution.

***Dr Worell:** Treatments for ectoparasites include injectable or oral ivermectin (Ivomec) dosed at 0.3 to 0.6 mg/kg on a weekly to biweekly basis as long as is needed. Weekly topical application of Lym Sulfa (DVM Pharmaceuticals, Miami, Fla) dip at the concentration of 1 part dip to 32 parts water is also used until the problem is resolved. In the case of Demodex mites in hamsters, Mitaban (Pharmacia & Upjohn, Bridgewater, NJ) can be applied topically, in addition to the above treatments. Removal of offending cagemates or objects in the environment that the animal is rubbing against may also be necessary.*

Q. Is skin biopsy necessary or more cost-effective than doing scrapings, cultures, etc, and then proceeding to a biopsy?

***Dr Morrisey:** It depends on the situation; skin biopsies often give excellent information regarding the cause of the dermatitis. Biopsy samples can also be used for the culture, and they often give more accurate results than a topical culture.*

***Dr Worell:** I usually perform a skin scraping and use as my next diagnostic test and the animal's response to treatment. I find that most pet rodent owners decline to have a skin biopsy performed.*

Q. Discuss the options for therapy if the owner won't spend money.

***Dr Morrisey and Dr Worell:** Therapy would be determined by what I considered the most likely cause to be. Ivermectin or antibiotics (topical or systemic) should be considered to treat a primary or secondary dermatitis.*

Marine Aquarium in a Restaurant

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Answers

1. The most likely diagnosis for the disease outbreak in this aquarium is a parasitic disease. The most frequently recognized external parasite of captive marine fish is the ciliated protozoan, *Cryptocaryon irritans*, which produces white spots on the skin and gills of affected fish. The irritation to the skin and gills results in the clinical signs of flashing (rubbing on items in the aquarium) and increased gilling behaviors.
2. A skin scraping would be indicated to confirm the diagnosis of *Cryptocaryon irritans*. The diagnostic features of the organism on a wet-mount skin scraping include a large (160-454 µm) oval-to-spherical ciliate protozoa (trophont) that moves in a slow, circular fashion. The organism has uniformly distributed cilia and a lobed macronucleus that appears to be horseshoe shaped.
3. Copper sulfate treatment is usually effective in controlling this disease in marine fish. The addition of copper sulfate to the aquarium water at a concentration of 0.18 to 0.22 mg/L for 14 to 21 days is usually effective. Lowering the salinity (ie, 20 parts per thousand) to create an osmotic shock to weaken the parasite may also aid in ridding the aquarium of *C irritans*.

FROM THE LITERATURE

Propofol as an Anesthetic for Tree Snakes

Shawn Messonnier, DVM

Propofol was tested in nine wild-caught brown tree snakes. A dose of 5 mg/kg was administered as an intracardiac injection. The mean duration of anesthesia was 24 minutes. The duration of anesthetic effect was shortened if the snake was agitated at the time of injection or had increased fat stores. Eight of the snakes experienced self-limiting apnea that lasted approximately 30 to 60 seconds immediately after the injection. Two of the snakes failed to achieve a plane of surgical anesthesia. There were only mild changes in heart rate, cardiac blood gas values, Spo_2, and $ETco_2$.

Anderson N, Wack R, Calloway L, et al: Cardiopulmonary effects and efficacy of propofol as an anesthetic agent in grown tree snakes, *Boiga irregularis*. *J Assoc Rept Amphib Vet* 1999.

Editor's Note: The authors describe the use of the new injectable anesthetic, propofol. Although intracardiac injection was used, they suggested that an injection could also be easily made in the tail vein in snakes with easily accessible ventral tail veins. Since propofol has a large volume of distribution, this results in a rapid drop in drug concentration as the infusion is discontinued. This allows for short anesthetic procedures after a single injection if a continuous infusion is not used. Since reptiles usually experience prolonged inductions and recoveries with many anesthetic regimens, the properties of propofol are particularly attractive for use in reptile medicine. Drug disposition is quite variable depending on the species, and propofol would be expected to act differently in different reptile species. This report sheds light on its use in at least one species of snake.

FERRET FACTS

Insulinoma in Ferrets

Michael A. Dutton, DVM, Dipl ABVP–Companion Animal Practice

Occasionally a mature ferret might start to exhibit sporadic weakness. The weakness is usually prominent in the rear legs of the ferret. One of the differential diagnoses that your veterinarian should include in the list of causes for this is an insulinoma. An insulinoma is a type of cancer in the beta cells that secrete insulin in the pancreas. This single (or multiple) tumor over-produces insulin. This results in a lowering of the blood glucose level. Diagnosis can be difficult because the body of a ferret has the ability to quickly raise its blood sugar level in times of stress or low blood sugar. By the time the ferret is seen by your veterinarian, the blood sugar level may be back to normal.

Diagnosis is routinely made based on the clinical signs, the age of the ferret and the ferret's response to orally administered sugar (eg, corn syrup). If, during one of these weak episodes, the ferret returns to normal quickly after you rub corn syrup on its gums (do not try to have the ferret swallow the corn syrup), a tentative diagnosis of low blood sugar can be made.

History and Diagnosis

Insulinoma usually has a slow onset. Signs include increasing weakness, lethargy, and hypersalivation. Rarely will the blood glucose level be so low to cause seizures. Ferrets can show rear limb weakness that mimics spinal cord disease. The blood glucose level will fall under 80 mg/dL. To diagnose, the clinician can test insulin levels. Anything over 350 pmol/L with hypoglycemia is suspicious.

Treatment

Treatment consists of both a medical and a surgical option. Surgical treatment consists of excising the affected pancreatic tissue. In cases of solitary tumors this works well, although recurrence of the tumor is likely. Multiple pancreatic tumors are not treatable by surgery and must be managed with medicine.

Medical treatment consists of lessening the effects of the high insulin level or increasing the blood sugar. Prednisone will cause blood sugar elevation and works for most ferrets. Another medication, diazoxide, works by inhibiting the effect of insulin on the cells. Administration of either of these medications would be lifelong. Prednisone is inexpensive, whereas diazoxide is costly. Some ferrets need both medications to control their low blood sugar. Unfortunately, most ferrets do not have a long life after insulinoma is diagnosed.

Client Teaching Guide

Reptile CARE SHEET

Thomas Ryan, DVM, Dipl ABVP–Avian Practice, Feather, Scales and Tails Veterinary Hospital, Westminster, Md

Jackson's Chameleon (*Chamaeleo jacksoni*)

✔ Subspecies

- *C jacksoni jacksonii* comes from Mt Kenya in Kenya, Africa. It is not exported. This subspecies is dark green and intermediate in size.
- *C jacksoni merumontana* comes from Mt Meru in Tanzania, Africa. It is the smallest subspecies, browner in color. Females may grow a single horn from the snout. This chameleon is rarely exported.
- *C jacksoni xantholophus* is also from Mt Kenya. It is also known as the "yellow crested" chameleon. Males have yellow and/or green along the sides and a blue tail. This subspecies is the type of Jackson's chameleon most frequently kept as a pet.

✔ Environment

These chameleons are territorial and prefer individual cages, approximately 2 × 2 × 3 ft. The cages need good ventilation, multiple branches, and plants to provide hiding spaces. Chameleons must have a climbing area so they can adjust their body temperature. They need basking light and a full spectrum light. Drinking water is obtained from the moisture on leaves. Cage humidity should be 80%, with a daytime temperature of 75°F to 78°F (23.8°C-25.6°C) and a nighttime temperature 8°F to 10°F cooler.

✔ Diet

The diet of the Jackson's chameleon is omnivorous. These lizards eat crickets, meal worms, and super worms. Once a week, they should be offered a variety: waxworms, grasshoppers, etc. All live prey needs to be gut-loaded.

✔ Breeding

The female Jackson's chameleon is receptive when she attains a light color. The female should be brought to the male's cage. Copulation lasts 10 to 45 minutes. If the female attempts to bite the male, remove her from the cage and try again in a few days.

The female will have a darkened skin pattern and will become more rotund over a period of 4 to 10 months depending on the environmental temperature and the time of year the pregnancy occurs. Female chameleons are ovoviviparous. The babies should be removed from the cage after the birthing process is complete. Neonates are 1 to 1½ inches long. They should be housed at 77°F (25°C) with 65% humidity. Babies are fed pin-sized crickets, fruit flies, etc.

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CASE REPORT

Foreign Body in a Dove

Amy B. Worell, DVM, Dipl ABVP–Avian Specialist

A 10-year-old female pet dove was examined for a 1-month history of decreased weight-bearing on the right leg, with an accompanying limp, and a 1-day duration of blood in the stool. A physical examination demonstrated that the bird was bright and alert but thin (264 g), with a moderate amount of dyspnea. Fresh blood was present in the stool. No abnormalities were observed on palpation of the body, although it was noted that the bird placed most of its weight on the left leg. Radiographs demonstrated no abnormalities in the skeletal system but a heavy metal density that had both straight and curling aspects to its form. It could not be discerned whether the metallic structure was in the intestinal loops or free in the abdomen. A blood panel and cloacal culture were the only other diagnostic tests permitted by the owner. Hospitalization was declined and the pet was sent home with metronidazole (10 mg/kg bid) and enrofloxacin (15 mg/kg bid) to be given orally, in addition to injectable Calcium EDTA (35 mg/kg bid). Symptomatic medication for the limp was not prescribed because the metallic density and blood in the stool were given priority for treatment.

The results of the blood work and culture were within normal limits. A recheck was recommended in 1 week.

The client returned in 2 weeks for a recheck exam. The pet was doing better in that no blood was noted in the stool, but the bird was not eating well and was still not using its right leg normally. Repeat radiographs demonstrated the metallic density to be similar in appearance to the previous radiographs. An additional week of calcium EDTA was dispensed, and the pet was sent home with hand-feeding formula to be fed twice daily.

The pet returned 2 weeks later for a follow-up examination. The bird had gained more than 30 g (new weight, 298 g) and was doing well at home according to the owner, even though the limp was still present. Repeat radiographs demonstrated no change in the heavy metal density. Because the pet was stable at this point and no change had been noted in the density, a barium series or exploratory surgery were recommended to the owner. The owner elected no additional treatment at this time.

The bird was brought in 4 months later exhibiting listlessness, a marked weight loss (now 216 g), a 1½-month history of eating only popcorn, and intermittent pus dripping from the cloaca. Radiographs demonstrated loss of detail in the coelomic cavity with the continued presence of the metallic foreign body. Additional diagnostics performed at that time demonstrated blood panel abnormalities: a white blood cell count of 58,000, toxic heterophils, rod-shaped bacteria noted on cytology of the cloacal discharge, and *Escherichia coli* cultured on a cloacal culture. The bird was started on injections of piperacillin (50 mg/kg bid) and amikacin sulfate (Amikin, Bristol-Myers Squibb; 10 mg/kg bid). Hospitalization and an exploratory surgery were declined.

A week later on recheck examination, the pet's body weight had improved (273 g), and pus was no longer dripping from the vent, but the bird was once again dyspneic on handling. Repeat blood work demonstrated a white blood cell count of 34,000. Antibiotic therapy was continued.

A recheck examination 2 weeks later demonstrated that the bird doing much better, with a white blood cell count of 13,000. The owner was considering allowing exploratory surgery at this time.

Two weeks later, the bird was brought for the surgery. The pet was anesthetized with isoflurane and oxygen (by mask), and an exploratory surgery was performed. A piece of wire was located outside of the gizzard adhered with fibrous tissue to the serosa covering the gizzard. The rest of the coelomic cavity appeared grossly normal. The abdominal wall and skin were closed with absorbable suture material. The pet recovered uneventfully from the surgery.

It is surmised that the pet ingested a piece of stainless steel wire that was used by the owner in making jewelry. It is thought that the wire was initially in the gizzard and migrated out, resulting in an infection in the intestinal loops and coelomic cavity.

Availability of Back Issues

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Q&A

Editor's Note: This month's question is taken from Pratt PW: *Review Questions and Answers for Veterinary Boards: Ancillary Topics,* ed 2. St Louis, Mosby, 1998.

Which rodent species is best bred in monogamous pairs?

A. rat

B. gerbil

C. hamster

D. rabbit

E. guinea pig

(B.) Gerbils mate for life and seldom accept a new mate, even when one dies. Males regularly groom and help care for the young. In other species, males are indifferent towards the young and may even harm them.

UPCOMING MEETING

American Veterinary Medical Association's 137th Annual Convention, Salt Lake City, UT; July 22-26. For information, call (847) 925-8070.

Readers: We welcome your questions, practice tips, and case reports. Please submit any materials to Susan Sibiski, 526 Cole Ln, Baltimore, MD 21220; ssibiski@home.com.

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