Journal of the American Association for Laboratory Animal Science
Vol 45, No 1
January 2006
Pages 57–63

Comparison of Rectal, Microchip Transponder, and Infrared Thermometry Techniques for Obtaining Body Temperature in the Laboratory Rabbit (*Oryctolagus cuniculus*)

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This study compared rabbit rectal thermometry with 4 other thermometry techniques: an implantable microchip temperature transponder, an environmental noncontact infrared thermometer, a tympanic infrared thermometer designed for use on humans, and a tympanic infrared thermometer designed for use on animals. The microchip transponder was implanted between the shoulder blades; the environmental noncontact infrared thermometer recorded results from the base of the right pinna and the left inner thigh, and the tympanic infrared thermometer temperatures were taken from the right ear. Results from each technique were compared to determine agreement between the test modality and the rectal temperature. The practicality and reliability of the modalities were reviewed also. According to this study, the implantable microchip transponder measurements agreed most closely with the rectal temperature.

Abbreviations: BMDS, Bio Medic Data System

Body temperature is a fundamental parameter in assessing an animal's health status. A core body temperature is the measurement of the deep body sites or hypothalamus.[10] Although the core body temperature is the true standard, these measurements require invasive procedures such as intra-abdominal radiotelemetry,[8] pulmonary artery catheterization, esophageal probes, and urinary bladder catheterization.[9,10] In clinical settings, rectal thermometry is the most common method for obtaining animal body temperatures; although this technique requires restraint and handling, it is considered minimally invasive. Implantable microchip transponder thermometry methods and infrared thermometer technology are becoming common practice.

The implantable microchip transponder uses an internal frequency generator that is built into the transponder.[2] A thermistor determines the frequency in the frequency generator. The thermistor is a variable resistor whose value changes proportionally with the temperature.[2] The frequency counter within the transponder counts the generated pulses a set number of times, and these individual counts are emitted from the transponder to the reader unit. The reader unit discards the highest and lowest counts and averages the rest of the measured pulses. The average frequency reading then is applied to an algorithm in the reader unit to display the temperature value.[2]

Infrared thermometry measures surface temperatures[11,15] and has the advantages of speed, accuracy,[11] and noncontact capability. Because all matter with temperatures above absolute zero emits infrared radiation, this technology measures the characteristic radiation of an object and converts it into a temperature value.[15] Characteristic radiation is picked up by the optical system, which focuses this energy onto a detector. The optical system is either a mirror or lens optic, depending upon the particular wavelength ranges of interest.[15] The detector then converts the radiation into an electrical signal that is emitted as a temperature value.[15] The laser sighting on the noncontact infrared thermometer allows the user to aim the device, and some laser sightings display the diameter of the measured area.[15] The laser actually plays no part in the temperature measurement.

The tympanic infrared thermometers use the same infrared technology as do the noncontact methods. The tympanic infrared thermometers are designed to be placed into the ear canal to read the infrared energy emitted by the target area. The ideal target is the tympanic membrane because it is anatomically close to the hypothalamus, which may give this modality a more representative core body temperature. Both of the tympanic thermometers used in this study were calibrated against the blackbody setting.[15] The blackbody is a precisely defined infrared signal used to calibrate and verify instant infrared ear thermometers.[15] The displayed temperature value has an added clinical bias (added value) established by the manufacturer for accuracy.

Previous studies have compared the implantable microchip transponder with rectal temperatures in marmosets,[6] mice,[4,12,19] rats,[12] goats, horses, and sheep.[10] The results suggest that these temperatures were comparable, with the exception of those for goats, horses, and sheep. Tympanic temperature studies also have been conducted on dogs,[9,17] cats,[15,17] mice, rats, guinea pigs, and rabbits.[17] Only the guinea pig transponder results differed significantly from rectal temperatures.[17] To date, the present study is the only one that compares measurements from the implantable microchip system, environmental noncontact infrared thermometer, human tympanic infrared thermometer, the animal tympanic infrared thermometer temperatures with digital rectal temperatures in laboratory rabbits. Although readings from an infrared tympanic thermometer designed for use on humans have been compared with rectal thermometer

Received: 9 June 2005. Revision requested: 17 Aug 2005. Accepted: 22 Aug 2005.
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temperatures in rabbits,[17] its ease of use has not yet been compared with that of the modality designed for use on animals. The objective of this study was to determine agreement between measurements from these newer thermometry modalities and rectal temperatures in the laboratory rabbit. We also discuss the practical clinical aspects of each thermometer.

Materials and Methods

Animals. This study used 46 male (weight, 3 to 4.5 kg) research-bred New Zealand White rabbits (*Oryctolagus cuniculus*) from Charles River Laboratories (St. Constant, Quebec, Canada), Covance Research Products (Denver, PA), and Harlan (Indianapolis, IN). They were serologically negative for *Treponema cuniculi*, *Encephalitozoon cuniculi*, cilia-associated respiratory bacillus, *Clostridium piliforme*, parainfluenza 1 and 2, reovirus, and rotavirus. They were culture-negative for *Pasteurella multocida*, *Salmonella* spp., *Bordetella bronchiseptica*, *Pasteurella* spp., *Clostridium perfringens*, and *Pseudomonas aeruginosa*. The rabbits were free of ectoparasites, hepatic coccidia, metazoan, intestinal coccidian, and other protozoa by external and fecal examination.

This study was conducted under an institutional animal care and use committee-approved protocol at a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International. The rabbits were housed indoors and maintained and handled in accordance with the *Guide for the Care and Use of Laboratory Animals*[13] and the institute's animal care policies. The animals were acclimated for a minimum of 48 h. The rabbits were single-housed in perforated-bottom caging (ComfortCage, Lenderking Caging Products, Millersville, MD) and fed a high-fiber diet (2031 High-fiber Rabbit Diet, Harlan, Indianapolis, IN) or 1% cholesterol diet (Bio-Serv, Frenchtown, NJ). Pulp-type bedding (Tek Fresh, Harlan, Indianapolis, IN) was placed in the cage pan beneath each cage. The drinking water was chlorinated at 6 to 7 ppm. A variety of commercial food and toy enrichments were used in their husbandry and care program.

The rabbits in this study were enrolled concurrently in other experimental protocols not known to affect temperature. Each rabbit was used as its own control. This concurrent use of the rabbits met the "3 Rs" of research by reducing the total number of rabbits used at this institution.[7,16]

Thermometry. Normal healthy rabbits were selected for measurement in this study, and no attempt was made to manipulate the internal temperature of the rabbits. Each rabbit received a gross otoscopic examination of both ear canals prior to temperature measurements to confirm normal ear anatomy and to document potential obstructions that might interfere with tympanic thermometry. Microchip transponders were implanted subcutaneously between the shoulder blades on the same day as the otic examination. After at least a 5-d waiting period, rabbit temperature measurements were collected over 4 separate days. The ambient room temperatures ranged from 19.4 to 21.1 °C, with an average of 20.0 °C over all collection days.

The rabbit temperatures were taken with a digital rectal thermometer, an implantable microchip transponder, an environmental non-contact infrared thermometer, and 2 tympanic infrared thermometers, one designed for use on humans and the other designed for use on animals. All thermometers were used and programmed according to the manufacturer's instructions. In addition, all the thermometers used in this study were calibrated by the manufacturer. Temperature measurements were collected in duplicate, and the sequence of thermometry was randomized for each rabbit. The randomization was restricted so that the same type of measurement was never collected twice in a row on the same animal. All 10 measurements for this study (one duplicate for each of the 5 measurement types) were made on a single rabbit before moving to collect measurements from the next rabbit. The duration to complete the sequence of 10 measurement procedures on a single rabbit ranged from approximately 5.5 to 9.5 min. The 46 rabbit temperatures were collected over 4 nonconsecutive days. The rabbits were placed on a stainless steel procedure table for all manipulations.

The rectal digital thermometer (Digital Fever Thermometer, Becton-Dickinson, Franklin Lakes, NJ) operated within a range of 32.2 to 42.2 °C with an accuracy of 0.2 °C. The manufacturer-listed measurement time was 1 min. The rectal probe with a cover was lubricated and placed approximately 1 cm into the rectum. The handler restrained the rabbit by using one arm to cradle it against the handler's body; the handler then lifted the animal's tail with the same hand. The tail was released after the thermometer was inserted into the rectum. An audible signal was emitted when the thermometer registered a final measurement.

The implantable microchip transponder (IPTT-200, Bio Medic Data System [BMDS], Seaford, DE) has an operating temperature range of 32 to 43 °C and an accuracy of approximately 0.5 °C. The skin over the injection site was prepared with isopropyl alcohol, and then it was pinched into a tented position for the subcutaneous insertion of the transponder. The transponder was placed in the region between the shoulder blades by using the manufacturer's 12-gauge introducer. The skin over the puncture site was pinched for a few seconds after removing the applicator. For this process, one person cradled the rabbit against his body, and a second person used both hands to implant the transponder. The transponder was tested for its identification prior to and after implantation before returning the rabbit to its cage. The transponder reading was taken with the Pocket Scanner System DAS 5007 (BMDS) and verified with the BMDS Notebook System DAS 5003 (BMDS) for confirmation of any abnormal readings. The manufacturer's stated reading distance is 50 mm, with a reading time of 20 ms.

In this study, 3 types of noncontact infrared thermometers were reviewed: 1) an environmental noncontact infrared thermometer (Raytek Rayngers ST, Santa Cruz, CA); 2) a tympanic infrared thermometer (Braun Thermoscan One-Second Thermometer, IRT-3520, Boston, MA) designed for use on humans; and 3) a tympanic infrared thermometer (Pet-Temp, Advanced Monitors Corp., San Diego, CA) designed for use on animals. The environmental noncontact infrared thermometer has an operating range of –30 to 400 °C with an accuracy of 1% and can take an instant measurement at a distance of 1.83 m. By using this thermometer, temperatures were collected from 2 locations on the rabbit. From a distance of approximately 1 m, the laser sight was aimed at the medial aspect of the base of the right pinna and the medial aspect of the left inner thigh. For these procedures, the rabbit was placed on a table and cradled with its left side against the handler's body. The rabbit then was lifted off the procedure table so that the left leg could be extended for the thigh measurement.

The tympanic infrared thermometer designed for use on humans in this study has a rated operating range of 10 to 40 °C with an accuracy of 0.2 °C and a measurement time of approximately 1 s. It has an adjusted temperature that is +0.6 °C over the black-body setting.[15] The tympanic infrared thermometer designed for use on animals has an operating range of 34 to 43 °C with an accuracy of 0.6 °C and a measurement time of less than 1 s. The displayed temperature has a manufacturer-calibrated clini-

Table 1. Temperature ranges and average temperature repeatability

Thermometry method	Temperature range (ºC)	Average temperature (ºC)	Repeatability variance	95% Repeatability limits	95% Agreement limits
Rectal temperature	38.1–40.8	39.3	0.215	±0.928	NA
Implantable microchip transponder	38.3–40.1[a]	39.0	0.038	±0.391	±1.48
Noncontact infrared thermometer, ear	25.8–34.7	31.7	2.05	±2.86	NA
Noncontact infrared thermometer, thigh	23.6–33.6	29.2	2.82	±3.36	NA
Human tympanic thermometer	36.7–40.3	39.1	0.275	±1.05	NA
Animal tympanic thermometer	32.2–40.5	38.7	1.32	±2.3	NA

NA, not applicable.
[a]Does not include outlying measurements.

cal bias (added value) so that it may be slightly higher than the blackbody temperature.[5] The appropriate respective lens covers were applied for each temperature measurement. For the tympanic infrared temperature procedures, the rabbit was placed on a table and cradled with its left side against the handler's body. The investigator held the distal portion of the right ear with the left hand and applied the tympanic thermometer with the right hand. The tympanic thermometers were placed into the ear canal with the probe pointing toward the left cheek of the rabbit. This orientation was intended to provide alignment of the temperature probe head to the tympanic membrane. An auditory signal acknowledges when the temperature was measured by each device.

Data analysis. Data were analyzed using both graphical and numerical methods described by Bland and Altman[1,3] for assessing equivalence in order to determine agreement. These methods were designed to determine how closely the results of the measurement systems agree. Agreement is a more appropriate standard of comparison than the historically more common technique of correlation and regression.[1,3] This analysis can be used to determine if a new technique agrees sufficiently to replace an established standard. For this study, the rectal temperature measurements were used as the standard for comparison with the results of the other thermometry modalities.

Prior to obtaining any measurements, this study's criteria for accepting that a proposed measurement system agreed with rectal thermometry were limits of agreement of less than ±2 °C (with 95% confidence). This criteria had a 2% probability of rejecting a truly equivalent method in quality to the rectal method. The rejection rate was used for planning the study, but it was not important for analyzing the results. The 2% rejection rate was estimated from a computer-based simulation conducted prior to collecting any measurements. The assumptions for the simulation included: 1) measurement variability would be no more than 10% as large as the variability associated with the temperature of a healthy rabbit; 2) the normal range for a healthy rabbit is 38.5 to 40.0 °C; 3) the normal range is assumed to be ±3 standard deviations in size; and 4) measurement variability about the true rabbit rectal temperature was approximately normal in probability distribution.

All 4 thermometry methods were evaluated using graphics. Agreement was calculated if the graphics met the statistical assumptions required for estimating agreement. Those that fell within this criteria were considered for agreement analysis by numerical methods.

The means, 95% limits for repeatability, and 95% limits for agreement (where appropriate) were reported. The means were intended to represent the average rabbit temperature by using a particular measurement method. The 95% limits for repeatability were intended to contain approximately 95% of the differences between pairs of results from the selected measurement method. The 95% limits for agreement were intended to contain 95% of the differences between paired measurements obtained by a specific pair of measurement methods. The 95% limits were calculated as 2 times the standard deviation for repeatability or agreement, as appropriate. Standard deviations for repeatability and agreement were calculated using established methods for estimating components of variance. All calculations and statistical graphics were produced using the R Language for Statistical Computing.[14]

Results

Animal examination. The otic examination showed that the external ear canals were clinically normal and unobstructed. Grossly, no noteworthy inflammatory processes were observed at the microchip transponder implantation site.

Temperature measurements. Overall, the temperature readings did not increase with restraint time. The temperature ranges, average temperatures, and 95% repeatability and agreement limits are listed in Table 1. The rectal and implantable microchip transponder produced the smallest ranges. Results in Figure 1A did not indicate any systematic differences between the implantable microchip transponder and rectal measurements, and the average difference between these measurements was approximately 0 °C. The average rectal temperature measurement and 95% repeatability limit for body temperature were 39.3 ± 0.928 °C. The average implantable microchip transponder temperature measurement and 95% repeatability limit for body temperature were 39.0 ± 0.391 °C. The 95% agreement limit between the implantable microchip transponder and rectal measurements was between ±1.48 °C.

Of the 46 microchip transponders implanted in this study, 4 failed to function. Malfunctions were confirmed with a separate reader unit. One of these transponders gave readings of 32.6 °C and 33.1 °C, whereas the normal rabbit body temperature range is between 38.5 to 40.0 °C.[18] This measurement was not included in the final analysis. Of the remaining 3 malfunctioning transponders, 2 of the transponders registered a verification reading after placement but did not register on the sampling date, although these transponders still could be palpated between the shoulder blades. The remaining transponder was not palpable and did not register a verification reading or a reading on the sampling date.

Both of the environmental noncontact infrared thermometer techniques produced the largest temperature ranges in this study (Table 1). These temperature readings were always lower than the rectal temperatures, and their measurements systematically differed from the average temperature (Figure 1B and 1C). These measurements did not meet the criteria for consideration of agreement to the rectal temperatures.

Neither tympanic thermometry system produced results that fit the criteria for consideration of agreement with the rectal temperatures. The tympanic infrared thermometers produced lower temperatures than the rectal thermometer measurements. In addition, their measurements were systematically different from the average temperature (Figure 1D and 1E); therefore numerical agreement between either modality and rectal thermometry was not estimated. The human infrared tympanic thermometer did produce more reproducible results, which were comparable to the rectal temperature, than did the animal infrared tympanic thermometer (Table 1).

Figure 2A to 2E provides direct visual comparisons between the modalities tested. All results for a particular modality are presented relative to rectal temperature measurements. The units for the *y* axis are the same in each of these plots. As in Figure 1, Figure 2 illustrates that the implantable microchip transponder had an average temperature difference of approximately 0 °C, compared with the rectal temperature. All other modalities had average temperature differences clearly less than 0 °C. In addition, the low variability of temperature differences from rectal thermometry relative to the other modalities is evident.

Discussion

Speed and accuracy are important when working with any animal. Rabbits are commonly used in research; they are a nervous prey species, and have a propensity for restraint-induced vertebral lumbar fractures. In addition, rabbits are commonly used in cardiovascular research, making them potentially less tolerant to distress after cardiovascular impairment from surgical or dietary manipulations. Noninvasive techniques and those that are rapidly accomplished for obtaining temperatures could contribute to improving their welfare in laboratories. Of the techniques tested, we consider the microchip transponder the most invasive because of the implanting process, but the temperature measurements do not necessitate restraining. All the tested procedures were accomplished rapidly, with the exception of the rectal digital thermometer, which required as long as 45 s to register a temperature measurement.

The environmental noncontact infrared thermometer was the most noninvasive technique evaluated in this study because it had a measuring distance up to 1.83 m. This noninvasive thermometry method could have contributed greatly to the welfare of laboratory rabbits when used as a component in their health status evaluation. The medial base of the pinna would be an easily accessible location without handling the rabbit, and the medial thigh was used as its contrast.

The environmental noncontact infrared thermometer thigh temperature results were the least comparable to the rectal readings and had the highest paired variability. This result was probably caused by interference from the hair covering the thigh. The high repeatable variance noted in the rabbit pinna temperature measurements may be attributed to the sampling sequence because rabbits were restrained slightly differently for each procedure. The thermometry modalities were randomized such that the pinna noncontact infrared measurement could be the first measurement or precede either a rectal, tympanic, or thigh noncontact infrared temperature measurements. For example, some rabbits had their head tucked in the axillary region of the restrainer for the entire 45 s that was required for measuring a rectal temperature. The restraint technique may have been a more important factor than originally anticipated, when using the environmental noncontact infrared thermometer on the ear. The combination of high repeatable variance and large difference from the rectal temperature results makes this modality unreliable for measuring rabbit body temperatures.

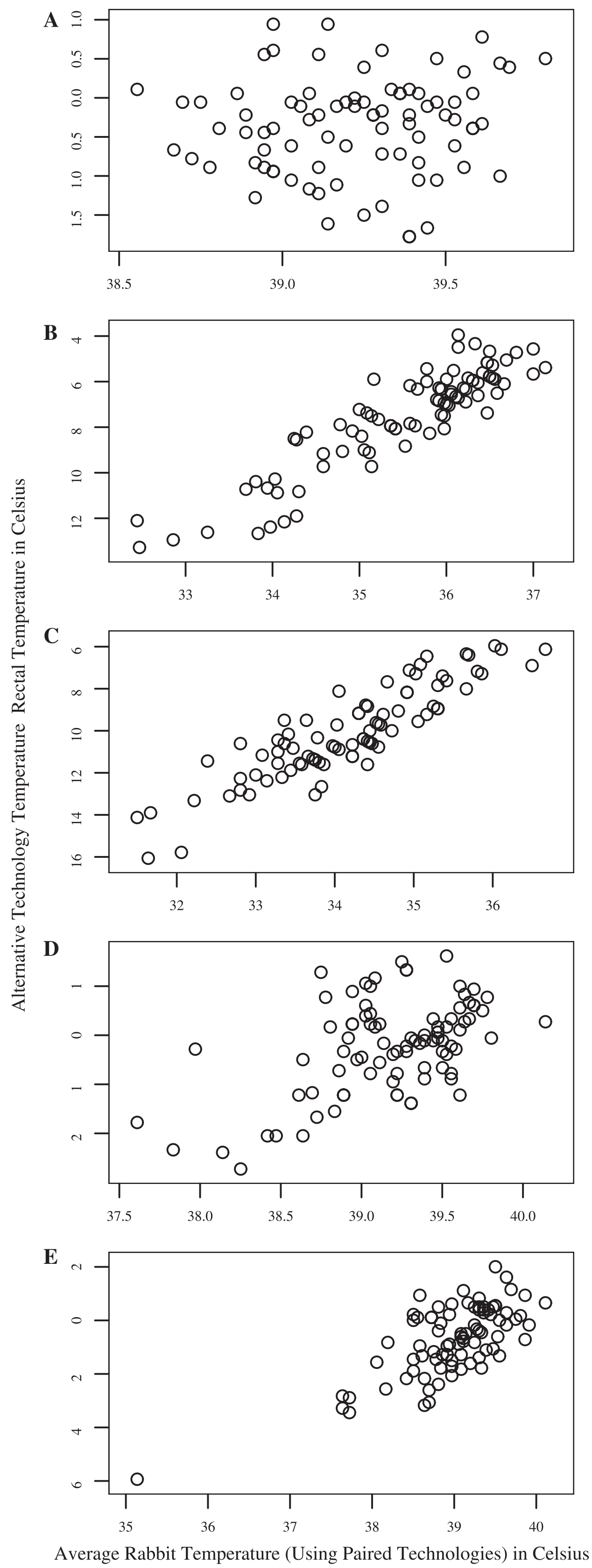

Figure 1. Agreement of temperature results from alternate technologies with rectal temperature. (A) Implantable microchip transponder. (B) Noncontact infrared thermometer on ear. (C) Noncontact infrared thermometer on thigh. (D) Human tympanic thermometer. (E) Animal tympanic thermometer.

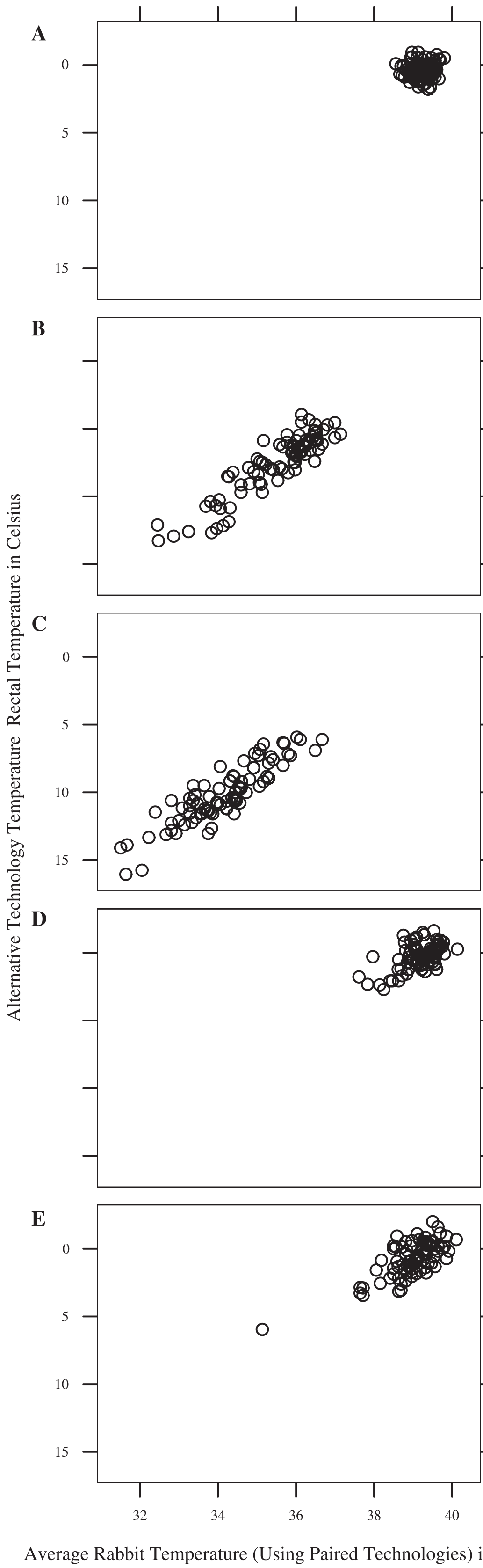

Figure 2. Pairwise agreement between rectal temperatures and temperatures obtained by alternative technologies. (A) Implantable microchip transponder. (B) Noncontact infrared thermometer on ear. (C) Noncontact infrared thermometer on thigh. (D) Human tympanic thermometer. (E) Animal tympanic thermometer.

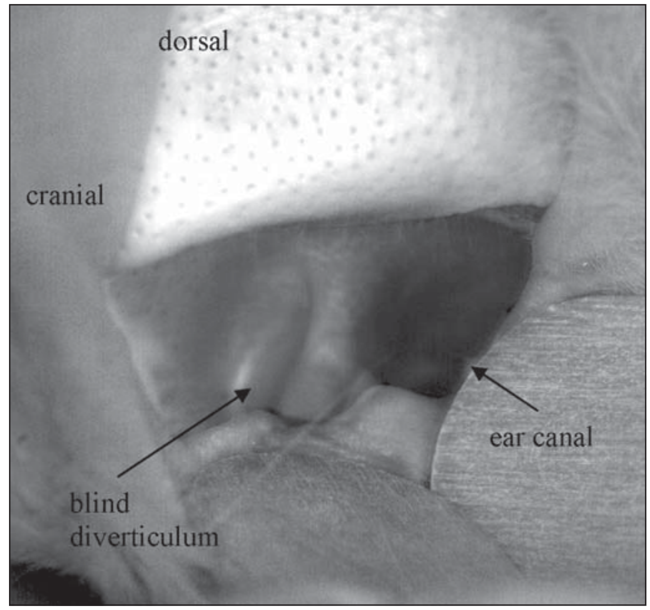

Figure 3. Interior view of the base of the pinna of a rabbit's left ear.

Most of the tympanic infrared thermometer temperatures were lower than the rectal temperature measurements. The temperature discrepancies may be attributed to the anatomy of the rabbit pinna. The base of the rabbit pinna has a blind diverticulum cranial to the ear canal. This diverticulum could easily be mistaken for the ear canal due to its proximity to the canal and the deep conical shape of the pinna base (Figure 3). Because it is more superficial, a diverticulum temperature reading is likely to be lower than the tympanic temperature. The lens position was the most important aspect of accuracy when using either of the tympanic infrared thermometers.[10,15]

When comparing the clinical aspect of each tympanic infrared thermometer, the system designed for human use was more user-friendly (Table 2). For example, the animal infrared tympanic thermometer lens cover was fragile and easily torn, and each measurement required a new lens cover. This system also required 7 to 14 s from the time the system was turned on to the time a temperature could be registered. The same lag time was required for additional temperature readings. The human tympanic thermometer did not have any noticeable delay from turning on the system to registering a measurement. In addition, the lens cover on this thermometer did not have to be changed between temperature measurements. This benefit is convenient for repeated recordings from the same animal, although soiled covers can interfere with temperature measurements. The human tympanic thermometer could be used as a rapid, noninvasive temperature screening system for rabbits.

The implantable microchip transponder method had the advantage of taking temperature measurements without handling the rabbit because one could record a temperature from a distance of 50 mm. According to the manufacturer, the lifespan of the transponders is indefinite because there are no batteries or moving parts. Other researchers have actually reused or recycled their transponders.[12] The present study did experience a failure rate of 8.7%. Although the implantable microchip transponder system was the most expensive per rabbit in this study, it was also the only system with a programmable capability up to 32 alphanumeric characters. The features of the implantable microchip transponder method would be beneficial for long-term studies, for studies in which frequent temperatures are

Table 2. Summary of thermometer attributes

Thermometer	Sampling time	Resample time	Advantages	Disadvantages
Digital rectal thermometer: B-D thermometer	≤45 s	≤45 s	-inexpensive	-long sample time -long restraint time
Implantable microchip transponder: BMDS IPTT-200 transponder	immediate	immediate	-programmable identification -no trauma from frequent sampling -noninvasive temperature measurements	-expensive start-up cost with programming and reader units -expensive per-transponder cost -8.7% failure rate noted in this study
Environmental noncontact infrared thermometer: Raynger ST	immediate	immediate	-noninvasive	-too variable and inaccurate at selected sample locations
Human tympanic infrared thermometer: Thermoscan	immediate	immediate	-inexpensive -lens cover reusable -no reset lag time -short restraint time -lens covers readily available	-accuracy depended upon position and skill -not rated for temperature measurements >40 °C[a]
Animal tympanic infrared thermometer: Pet-Temp	immediate	≤14 s	-designed for animal use -relatively short restraint time	-accuracy depended upon position and skill -long start and reset lag times -lens covers must be ordered -battery replaced by manufacturer

[a]Not rated for temperature measurements above 40 °C but it will display readings above this temperature.

required, where programmable information is useful, and in situations where rabbits are group-housed.

Our study did not compare these thermometry techniques outside of the normal rabbit rectal temperature range. All of the tested thermometers were rated for measuring above and below the normal rabbit rectal temperature range, except for the human tympanic thermometer, although it will register temperatures >40 °C.

In this comparison, only measurement results from the implantable microchip transponder showed acceptable agreement with measurements from the rectal system. Interestingly, repeatability variance for the implantable microchip transponder was smaller than that estimated for the rectal temperature. This result combined with agreement suggests that the implantable microchip transponder was a better system than the rectal method for measuring the body temperature. Of the tested methods, only the implantable microchip transponder measurements met the acceptance criteria for equivalence to the rectal temperature in the laboratory rabbit.

Acknowledgments

Special thanks are extended to the following: Patricia Nossov, Linda Davis, and Jeffery Arbenz (Division of Veterinary Medicine, Walter Reed Army Institute of Research/Navy Medical Research Center) for supporting the resources needed in this project; James Sheets and Dave Bentzel (Division of Laboratory Animal Medicine, Armed Forces Institute of Pathology) for providing access to the rabbits; Omar Feliciano, Angela King, and Aaron Jackson for their logistic support and expert handling skills; Alec Hail and Craig Harms for laboring over the editing.

Disclaimer: The rabbits were housed at the Armed Forces Institute of Pathology. This material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense. Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, NRC Publication, 1996 edition.

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