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Comparison of Rectal and Infrared Thermometry for Obtaining Body Temperature of Gnotobiotic Piglets in Conventional Portable Germ Free Facility

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ABSTRACT : Gnotobiotic piglets (n = 10) were hand-reared in conventional germ-free facilities. Piglet body temperatures were measured with rectal and non-contact infrared thermometry (NIFT) on the lower eyelid, auricular center and margin, parietal regions, axilla, central abdomen and dorsum, and the perianal region. Body temperature measurements at central abdomen, cranial dorsum, and perianal regions had NIFT values which had a significant linear relationship ($p<0.0001$) with rectal thermometry. The predicted equations of between-subject formulas were calculated as follows: rectal temperature, 28.07489+0.30372×central abdominal surface temperature; rectal temperature, 34.02799+0.15197×central dorsum surface temperature; and rectal temperature, 33.87937+0.15676× perianal temperature. These results suggested that NIFT could serve as a valid alternative to rectal thermometry in a portable germ-free facility without disturbing experimental animals. The development of a NIFT body temperature evaluation that does not require animal restraint is clinically advantageous, particularly in gnotobiotic piglets, and would be significantly less stressful for experimental procedures in germ-free facilities. (**Key Words :** Gnotobiotic Piglet, Rectal Temperature, Infrared Thermometry, Portable Germ Free Facility)

INTRODUCTION

The gnotobiotic laboratory animal has long served as a valuable tool for experimental study (Coates, 1975). In recent years, gnotobiotic pigs have been used in many types of research, including xenotransplantation (Chapman et al., 1995; Tucker et al., 2002). Accordingly, rearing systems and handling techniques specific to germ-free experimental animals and germ-free facilities have been developed with their increasing use. Live gnotobiotic pigs have been successfully delivered to conventional germ-free facilities, and aseptic manipulation of gnotobiotic pigs is essential during handling (Coates, 1975; Yuan et al., 1996; Tucker et al., 2002).

Germ-free swine can be routinely procured by both hysterectomy and hysterotomy procedures. Piglets can be obtained and transported to the laboratory or to incubators without contamination through the use of lightweight portable isolation equipment (Meyer et al., 1964). Germ-free facilities have been used in gnotobiology for a relatively short time period. Therefore, many of its characteristics have not yet been investigated. The production of gnotobiotic piglet, although well-vitalized, is complicated in execution, operation, and manipulation in portable germ-free facilities. Many stress factors may influence the experimental animal's physical status during manipulation and may therefore modify investigation results (Bayne, 1996).

Body temperature is a fundamental parameter in the assessment of animal health status. The core body temperature is the measurement of deep body sites or the hypothalamus (Goodwin, 1998). The core body temperature is the true standard, however, its measurement requires invasive procedures. Rectal thermometry is the most common method used in obtaining animal body temperatures in clinical settings. Although it is minimally invasive, it requires restraint and handling. The ideal measurement method for experimental animals would be accurate and would not require excessive contact, restraint, or handling.

Accordingly, infrared thermometer technology is

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becoming common in experimental practice. Non-contact infrared thermometry (NIFT) is an inexpensive and non-invasive method used to obtain body temperatures in experimental animals. Few studies have evaluated the accuracy of NIFT in gnotobiotic piglets in portable germ-free facilities, although several studies have described its use in mammals (Loughmiller et al., 2001; Saegusa and Tabata, 2003; Chen and White, 2006).

Gnotobiotic piglet production is currently well-vitalized but complicated in execution, operation, and manipulation in portable germ-free facilities. We assessed the reliability and accuracy of NIFT measurements at multiple locations on the body surface compared to rectal temperatures in gnotobiotic piglets.

MATERIALS AND METHODS

Gnotobiotic piglets

Crossbred piglets (York and Landrace) were randomly assigned to this study; 10 litters were included I the study. Animals were housed in accordance with protocols approved by the Seoul National University Institutional Animal Care and Use Committee (SNUIACUC). Closed hysterectomies were performed using a surgical isolator and an open caesarian section. Sows were injected intramuscularly with a Zolazepam and Tiletamine analgesic-tranquilizer (Zoletil®; Virvac, Korea) according to the manufacturer's instructions. A germicidal trap containing 80 litres of 5% chlorhexidine and water solution (v/v) was prepared in a conventional germ-free isolator-incubator system. Piglets were rapidly removed from the uterus in the sterile hysterectomy hood and were passed into an attached isolator through a plastic transfer sleeve. Surgeries were performed in three minutes or less to ensure live piglets. Individual staff members handled sow restraint, anesthesia, surgery, hysterectomy hood and isolator management, and the conventional germ-free incubator. The sow was humanely euthanized during the surgical procedure; sterile surgical packs, materials, tools, and equipment were sterilized and equipped prior to surgery.

Conventional portable isolators and incubators were constructed of stainless steel surrounded with polyvinyl canopies and were maintained under positive pressure. The floor area measured 60×120 cm, and was divided into four compartments by removable stainless steel partitions (Figure 1). Each pig was housed in a separate compartment with a floor area of 30×60 cm. Pre-filtered and pre-heated air was blown into the isolators from a centralized system; air was sterilized by passage through a fiberglass filter. The temperature was maintained at 35°C for the first 3 days and reduced to 23°C over a 7-day period.

Piglets were fed sterile milk substitutes from shallow sterile steel troughs. Pasteurized cow's milk diets (crude protein 4%, crude fat 5%, water 85%, crude fiber 0%; approximately 670 kcal/liter) were used along with a commercially available diet for orphan animals. Piglets were injected intramuscularly with vitamin E (34 I.U.) and selenium (0.8 mg) at the initiation of experiments. An iron-dextran compound (2 ml) containing iron (100 mg) as ferric hydroxide per ml was injected at 3 days of age. Milk was fed at a rate of 30 ml per feeding on day 1, and subsequent feedings were administered at 7.5 ml per feeding. All

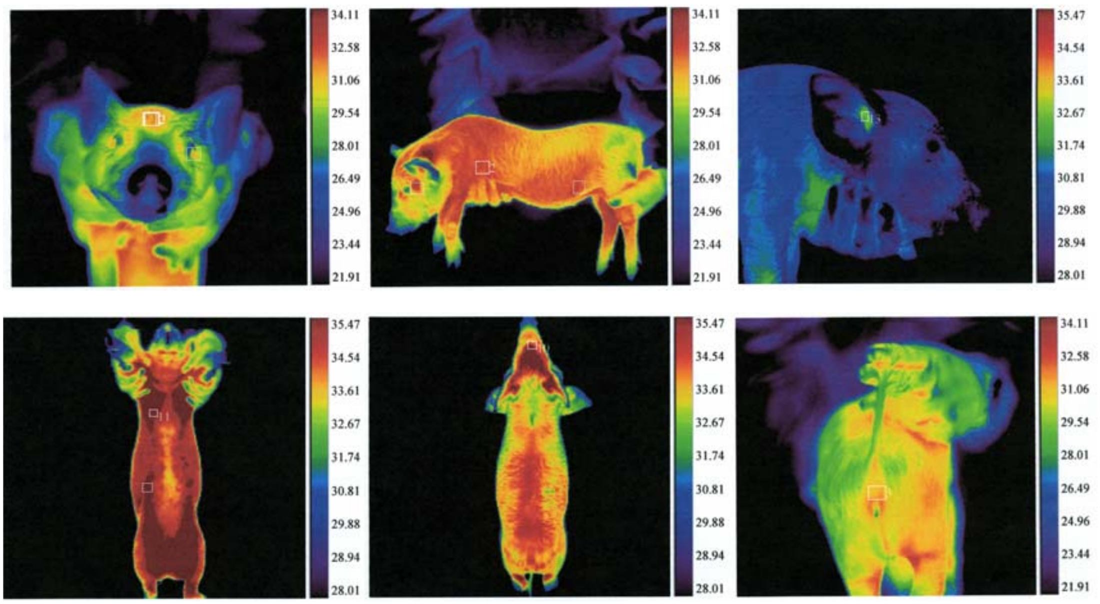

Figure 1. Thermal imagies of temperature gradations over piglet surface areas of the (a) parietal region, (b) auricular margin, (c) auricular center, (d) abdominal center, (e) center of the dorsum, (f) and the perianal regions. Selected positions for NIFT measurement are identified.

experiments were performed under germ-free conditions.

Body temperature imaging for site selection

A single piglet was selected after procurement, and body temperature imaging was conducted to select the NIFT target region. A side image of the patient's body was recorded with a thermal imaging camera (ThermaCAM E2 FLIR; FLIR Systems). The camera was aimed after an image preview with the inbuilt liquid crystal display; camera output was stored with a digital video recorder. Video images were used to measure the temperature of the skin body surfaces.

The thermal imaging camera defined settings were as follows: emissivity of pig skin, 0.98; reflected air temperature (ambient temperature in the abattoir at the point of measurement), 21°C; and the distance between the camera and the pig skin surface, 1.5 m. The temperature recording range was set between 16-44°C. The camera was operated in spot temperature mode to measure the temperature on the whole body surface (Figure 1). Several body points (auricle, ear canal, frontoparietal, infraorbital, axillar, dorsum, abdomen, anus resions) were chosen for analysis.

Thermometry

Temperatures were measured between 8-10 a.m. at ambient temperature of 21±1°C within a 2 week period. Temperatures were measured with a rectal thermometer and NIFT while piglets were free in a separate compartment; these measurements were taken simultaneously. The rectal thermometer (Digital Fever Thermometer; Becton-Dickinson, Franklin Lakes, NJ, USA) operated within a temperature range of 32.2-42.2 (±0.2°C). Rectal thermometers were lubricated and inserted approximately 6-7 cm into the rectum (Dollberg et al., 1993; Thoresen et al., 2001), and readings were taken when the thermometer beeped. NIFT used was a Raytek ST20 Pro (Raytek Raynger ST, Santa Cruz, CA, USA); this thermometer had a temperature range of 23-510 (±0.1°C) with a response time of <0.5 s and an optical resolution of 12:1. Optical resolution refers to the distance from the object and the size of the spot where the temperature was measured; a 12:1 optical resolution will measure a spot 3.8 cm in diameter when held 30 cm from the object. NIFT measurements and rectal temperatures were measured from 8 regions (auricle, ear canal, frontoparietal, infraorbital, axillar, dorsum, abdomen, anus resions) while the piglets were still. Measurement devices were held 30 cm from the target.

Data analysis

Descriptive statistics (mean and standard deviation) were calculated, and all statistical analysis was performed with the SAS package (SAS Institute, Inc., Cary, NC, USA). Regression methods were used to assess the correlation between rectal and NIFT temperatures. Predicted equation formulas were calculated using the coefficient of linear model. All tests were calculated under the 95% confidence level. Statistical significance was defined at p values <0.05.

RESULTS

Estimating the body temperature

Surface body temperature ranges were evaluated to select the experimental target regions; 8 sites were selected for NIFT-measurement sites, including the lower eyelid, auricular center and margin, parietal region, axilla, central abdomen, central dorsum, and the perianal region (Figure 1).

Thermometry

Linear regression analysis demonstrated a significantly linear relationship between rectal temperature and NIFT temperature values at the central abdomen, central dorsum, and the perianal region ($p<0.001$). Other regions such as the lower eyelid, auricular center and margin, parietal region, and the axilla region demonstrated no significant relationship. The residual analysis suggested a normal distribution, and therefore the linear regression equation was used for the coefficient determination to describe the relationship between rectal temperatures and abdominal/ dorsal/perianal NIFT values (Figure 2). Linear regression models predicted equations as follows: rectal temperature = 28.07489+0.30372×central abdominal surface temperature; rectal temperature = 34.02799+0.15197×central dorsal surface temperature; and rectal temperature = 33.87937+ 0.15676×perianal temperature ($p<0.0001$).

DISCUSSION

Speed and accuracy are essential with procedures involving experimental animals in germ-free facilities. Gnotobiotic pigs in germ-free facilities have been used in gnotobiology for a relatively short time period, and management of gnotobiotic pigs in such facilities require additional investigation. Rectal temperature measurement is a stress factor which could adversely affect the piglets. Repeated rectal temperature measurements and restraint could lead to nervousness in pigs and possible restraint-induced rectal injuries. Rapid and non-invasive temperature measurement techniques could contribute to improvements in animal welfare in laboratory conditions. The NIFT is a method that does not necessitate restraint.

Results from the present study suggested that NIFT is a reliable clinical device for approximation of the rectal temperature of gnotobiotic piglets reared in portable germ-free facilities, concordant with other mammalian studies. A

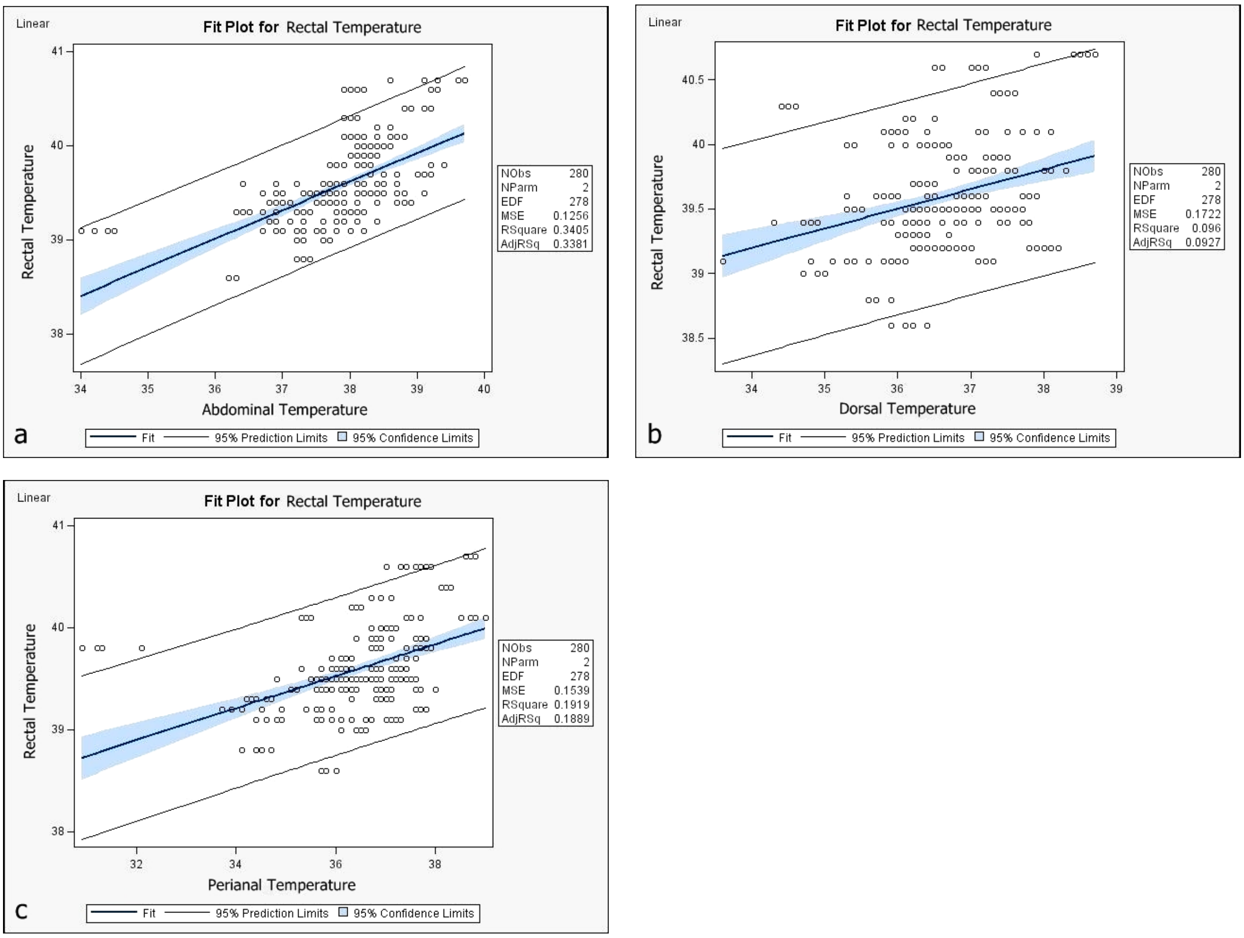

Figure 2. Regression analysis for the relationship between the means of all rectal temperatures and surface temperatures of (a) the abdomen, (b) the dorsum, and (c) the perianal area of all piglets. Simple linear regression analysis showed that there were significant correlations between NIFT measurements on 3 sites and rectal temperature in three sites. There were no significant correlations between NIFT measurements on other 5 sites (Data not shown).

study evaluated the ability of NIFT in normal pigs to detect rises in body surface temperature during a febrile response, and the authors concluded that the deviced detected temperature rises with accuracy. However, this study did not identify a correlation between NIFT temperature readings and rectal temperatures (Loughmiller et al., 2001). In a mouse study, body surface temperatures were obtained with contact infrared thermometry in multiple body locations, consistent with body temperatures (Newsom et al., 2004). Another study revealed that ear and back skin temperatures correlated well with rectal temperatures (Saegusa and Tabata, 2003).

NIFT measurement had a reasonable accuracy in detecting tympanic fever in children (Ng et al., 2005). We evaluated the NIFT readings comparing with the results of rectal temperatures using the regression analysis model (Greenes and Fleisher, 2001). Linear regression analysis is a standard method for evaluating correlation reliability between experimental group and control group data (Chatterjee and Hadi, 2006). The correlation between NIFT body surface temperatures with rectal temperatures was significant in the present study from the statistical analyses. Regression analysis revealed that only the three sites of NIFT thermometer location (abdominal, dorsal, and perianal) was a significant predictor of rectal temperatures. Central abdominal, central dorsal, and perianal area measurements were conditionally reliable indicators of rectal temperature with possible equation formulas in the present study. NIFT measured an area of 3.8 cm diameter located at 30 cm from the target in the present study. The proximity of the sensor to the skin in contact methods may also affect temperature by reducing the effects of hair on temperature readings. Body surface areas with less piglet hair allowed for more accurate NIFT measurements of skin body surface temperatures, as hair causes cooling effects on the skin. Acquisition of temperatures from a closer range may also increase the accuracy of rectal temperature calculation in regression analysis by decreasing the area measured.

In conclusion, the NIFT method is rapid, easy, lowstress, and inexpensive. Further, the measurements are valid and reliable in gnotobiotic piglets in portable germ-free facilities. NIFT thermometer can be performed without animal restraint, and may be the best option for clinical assessment of body temperatures in gnotobiotic piglets in portable germ-free facilities.

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