Use of chromametry and digital photography for objective measurement of skin color in clinically normal dogs

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Objective—To evaluate whether skin erythema in clinically normal dogs can be quantified by use of chromametry and image analysis of digital photographs.

Animals—9 German Shepherd Dogs and 10 mixed-breed dogs.

Procedure—Hair was clipped at 7 sites on the body. Skin erythema was evaluated at the axillary region, right and left lateral aspect of thorax, right and left loin area (ie, part of the back between the thorax and pelvis), right and left groin area (ie, the junctional region between the abdomen and thigh), metatarsal digital pad, and on the nose. Replicate measurements were done by use of chromametry and image analysis of digital photographs, using erythema values in accordance with the Committee International d’Éclairage (CIE)-Lab color system.

Results—Repeatability was high for both techniques. Within-dog variation was lower than between-dog variation. Between-dog variation was high for both groups of dogs. Interregional variation was significant in German Shepherd Dogs and mixed-breed dogs. Erythema values revealed symmetry between the right and left lateral aspects of the thorax and loin and groin areas.

Conclusions and Clinical Relevance—Precise objective methods are available for skin erythema quantification. Chromametric and photographic erythema values had a high within-dog reproducibility. Between-dog variability was high for German Shepherd Dogs and mixed-breed dogs as was regional variation, indicating differences in color among dogs. (Am J Vet Res 2002;63:559–564)

Skin color is important in the characterization of cutaneous disorders. Intensity of the color gives an impression of the stage of the pathologic process. Further, a change in color is a measure of therapeutic effect and as such is regularly used as an efficacy criterion in clinical trials.

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Chesney\textsuperscript{4} used an electronic thermometer to evaluate dog skin and coat temperature and a humidity meter to measure coat air humidity. Additionally he measured skin hydration in clinically normal dogs and in dogs with atopy or a scaling dermatosis.\textsuperscript{5} Using chromametry the effect of ultraviolet-irradiation on skin color has been assessed in dogs,\textsuperscript{6} and skin blood flow has been evaluated by laser Doppler flowmetry.\textsuperscript{7}

Of the morphologic features of skin, skin color appears to be one of the most commonly reported in clinical work. However, color intensity has so far been characterized subjectively, as for example in the intradermal skin test evaluation.\textsuperscript{11} The aim of the study presented here was to evaluate whether skin erythema in clinically normal dogs could be quantified by use of chromametry and image analysis of digital photographs.\textsuperscript{12}

Materials and Methods

Animals—Two groups of dogs were included in our study. Group I consisted of 9 German Shepherd Dogs (7 females and 2 males), and Group II included 10 mixed-breed dogs (6 females and 4 males). German Shepherd Dogs were chosen, because this breed is common among those admitted for dermatologic problems at the university clinic. All dogs were clinically normal pets between 1 and 10 years of age (median, 3 years). None of the dogs had a history of previous skin disease, and at physical examination no skin lesions were found on any of the dogs.

Hair was gently clipped at 7 sites of the body as follows: the axillary region, right and left lateral aspect of thorax, right and left loin area (ie, part of the back between the thorax and pelvis), and right and left groin area (ie, the junctional region between the abdomen and thigh). The clipping was done symmetrically. Areas of 5 X 5 cm were clipped at each site. Before measuring the skin color, a lag time of 20 minutes was given. If an erythematous rash was observed after clipping, another area was clipped next to the previous area. The metatarsal digital pad of the right hind limb and the nasal planum was cleaned but not rinsed.

Equipment—A chromameter,\textsuperscript{8} a lightweight handheld color analyzer for measuring reflected object colors, was used to measure skin erythema. The chromameter yielded a erythema value, a so-called a* value, according to the Committee International d’Éclairage (CIE)-Lab color system.\textsuperscript{9} In this color system a color is expressed in a 3-dimen-
sional coordinate system with an L-axis (brightness), a* -axis (green-red), and b*-axis (yellow-blue).

A digital camera\textsuperscript{9} equipped with a 105-mm Micro lens and a ring flash\textsuperscript{9} was used for photography. A turbo battery\textsuperscript{9} ensured a full-power flash discharge at every shooting. The shutter was preset at one eightieth of a second, the aperture on 32, and the flash on manual operation, half power. Focus was fixed to ensure that every photograph was taken at the same distance. All photographs were obtained at a 90° angle to the object. Image analysis of the photograph was performed, using a software program.\textsuperscript{9}

Evaluation of skin color—In the group-I dogs, skin color was measured consecutively 10 times bilaterally at the lateral aspect of thorax, loin area, and groin area. Triplicate measurements were made in the axillary region and on the nose and digital pad. The dogs in group II had triplicate measurements made in all regions. Whereas 10 repeated measurements were done to evaluate method precision, triplicate measurements were performed as the standard number of illuminations applied in chromametry to obtain 1 measure-

The evaluated skin sites represented areas regularly affected in skin disorders, the thorax being the common site for intradermal skin testing. Between individual measurements the chromameter was lifted from the skin, and photographs were taken at half-minute intervals. The chromameter was calibrated against the white standard calibration plate before each sequence of measurements. Prior to each series of photographic session an identification photograph and a standard gray card photograph were taken. To ensure that measurements were made of the same area every time, the area was marked with a felt pen. Dogs were held in the same position during examination of each site.

The same clinician made all measurements. Dogs in group I were evaluated in their owners’ homes under indoor light conditions. All measurements on dogs in group II were done in the university clinic under equal light conditions. A photometer\textsuperscript{10} was used to ensure that ambient light had no effect on the photographic acquisition regardless of location. The ambient temperature was recorded.

Image analysis—Following image acquisition, photographs were analyzed using computer software.\textsuperscript{11} Color adjustment was made by use of the standard gray card photographs. Mean red, green, and blue values of a fixed area on the photograph were obtained and converted into erythema values, quasi-a* values. The area size for analysis was the same as that by chromametry (ie, 50 mm\textsuperscript{2}). Equations described by Takiwaki and coworkers\textsuperscript{12} were used to calculate the estimated erythema value, quasi-a* value, corresponding to the a* value from the CIE-Lab color system. Prior to measurements, the use of chromametry and digital photography were compared in the clinic using red color plates.\textsuperscript{12} A significant positive linear correlation (r = 0.68) between chromametric values and values from image analysis of digital photographs was found.

Data analysis—Method repeatability (precision) of the 2 techniques was evaluated by calculating mean, SD, and percent coefficient of variation (CV%) of the 10 consecutive measurements. These summary measurements were used, as they were raw data. The mean and mean CV% were used as an overall measure of variation. Within-dog and between-dog variation was assessed for measurements.

The relationship between the 2 techniques was estimated, using a regression analysis of chromametric (a*) on photographic (quasi-a*) erythema values. The amount of variation over the range of quasi-a* values was determined by use of a residual plot\textsuperscript{13} of the difference between observed chromametric a* and predicted chromametric a* values versus quasi-a* values. Further, the correlation of erythema values was calculated on the basis of mean values for each dog, separately for both groups of dogs.

Regional differences in erythema were evaluated by mean and SE values of the 6 regions. An ANOVA was used to differentiate the sources of variation: dogs, sex, and region. Test for symmetry was made by comparing measurements on right and left side of the body (paired t-test). All significance tests were 2-sided, and the level of significance was set at P < 0.05.

Results

The CV% was calculated for each region in every dog, and a mean CV% gave the overall estimate of method repeatability (Tables 1 and 2). Both techniques had CV% < 10%. One dog had negative erythema values, and data from this dog were left out from calculations to avoid a falsely high CV%. Another dog had several missing values, and data from this dog were excluded from the calculations (data not
shown). A general picture of a low within-dog and between-dog variation was seen. For chromametry, the mean between-dog variation for the lateral aspect of the thorax and loin and groin areas was 29.7% (range 17.9 to 39.0%) in German Shepherd Dogs and 45.0% (range 26.0 to 60.4%) in mixed-breed dogs. For digital photography, the mean between-dog variation for the lateral aspect of the thorax and loin and groin areas was 34.9% (range 7.5 to 51.1%) in German Shepherd Dogs and 52.1% (range 22.4 to 75.0%) in mixed-breed dogs.

A plot of the agreement between technically derived erythema values in group-I and -II dogs revealed SD of residuals of 1.6 and 1.8, respectively, indicating that prediction of chromametric erythema values from photographic erythema values may be 3.3 to 3.5 below or above the observed chromametric value (Fig 1 and 2). Variation of data were equal throughout the range of quasi-a* values. The correlation (r) between chromametric and photographic erythema values was 0.9 for German Shepherd Dogs and 0.7 for mixed-breed dogs.

Regional differences in skin erythema were found with both techniques in both groups of dogs (Fig 3 and 4). Results of paired t-tests confirmed that the lateral aspect of the thorax, axillary region, and loin and groin areas had comparable mean erythema values. Chromametric erythema values for the nose and digital pad were significantly lower than the other regions.

In German Shepherd Dogs a substantial percentage of the data variation was the result of regional differences (chromametry, 86.5%; photography, 70.1%). Of the total variation, a small percentage was the result of differences between dogs (chromametry, 2.9%; photography, 7.1%). In mixed-breed dogs the variation between regions and between dogs was substantial (regions: chromametry, 44.3% and photography, 28.7%; between dogs: chromametry, 28.9% and photography, 32.5%).

No significant differences were observed between right and left side measurements in either group (Fig 5). Test for effect of sex on data variation was not possible. In mixed-breed dogs the variation between right and left side measurements was equal.

### Table 1—Repeatability of 10 consecutive erythema values obtained by use of chromametry from the right and left lateral aspect of the thorax and loin (ie, part of the back between the thorax and pelvis) and groin (ie, the junctional region between the abdomen and thigh) areas in 7 German Shepherd Dogs

<table>
<thead>
<tr>
<th>Region</th>
<th>Mean</th>
<th>95% CI</th>
<th>Mean SD*</th>
<th>Mean CV%†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thorax</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>3.5</td>
<td>2.5–4.6</td>
<td>0.3</td>
<td>8.1</td>
</tr>
<tr>
<td>Left</td>
<td>3.4</td>
<td>2.0–4.5</td>
<td>0.2</td>
<td>7.5</td>
</tr>
<tr>
<td>Groin area</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>8.3</td>
<td>6.7–10.0</td>
<td>0.4</td>
<td>5.2</td>
</tr>
<tr>
<td>Left</td>
<td>8.9</td>
<td>7.4–10.5</td>
<td>0.6</td>
<td>6.7</td>
</tr>
<tr>
<td>Loin area</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>3.6</td>
<td>2.6–4.6</td>
<td>0.2</td>
<td>6.7</td>
</tr>
<tr>
<td>Left</td>
<td>3.5</td>
<td>2.2–4.7</td>
<td>0.2</td>
<td>7.1</td>
</tr>
</tbody>
</table>

*Mean SD calculated from 7 SD of 10 repeated measurements on each dog. †Mean CV% calculated from 7 CV% of 10 repeated measurements on each dog.

Quasi-a* values = Erythema values determined by chromametry. CI = Confidence interval. CV% = Percent coefficient of variation.

### Table 2—Repeatability of 10 consecutive erythema values obtained by use of image analysis of digital photographs from the right and left lateral aspect of the thorax and loin and groin areas in 7 German Shepherd Dogs

<table>
<thead>
<tr>
<th>Region</th>
<th>Mean</th>
<th>95% CI</th>
<th>Mean SD*</th>
<th>Mean CV%†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thorax</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>17.0</td>
<td>9.4–24.6</td>
<td>1.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Left</td>
<td>17.9</td>
<td>12.0–23.8</td>
<td>0.7</td>
<td>4.9</td>
</tr>
<tr>
<td>Groin area</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>38.9</td>
<td>34.2–43.7</td>
<td>1.8</td>
<td>4.8</td>
</tr>
<tr>
<td>Left</td>
<td>36.9</td>
<td>34.2–39.8</td>
<td>1.4</td>
<td>3.6</td>
</tr>
<tr>
<td>Loin area</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>16.4</td>
<td>8.3–24.5</td>
<td>0.8</td>
<td>7.6</td>
</tr>
<tr>
<td>Left</td>
<td>15.0</td>
<td>8.3–21.8</td>
<td>1.0</td>
<td>9.0</td>
</tr>
</tbody>
</table>

Quasi-a* values = Erythema values determined by image analysis of digital photographs.

See Table 1 for key.

![Figure 1](link) — Plot of agreement between chromametric and photographic erythema values in 8 German Shepherd Dogs. Residual a* values (the difference between chromametric a* and predicted chromametric a* values) versus photographic quasi-a* values are plotted. Predicted chromametric a* values were obtained by a regression analysis of chromametric and photographic erythema values. The SD residual was 1.6, and the variation was equal throughout the range of quasi-a* values.

![Figure 2](link) — Plot of agreement between chromametric and photographic erythema values in 10 mixed-breed dogs. Residual a* values (the difference between chromametric a* and predicted chromametric a* values) versus photographic quasi-a* values are plotted. Predicted chromametric a* values were obtained by a regression analysis of chromametric and photographic erythema values. The SD residual was 1.8, and the variation was equal throughout the range of quasi-a* values.
Discussion

In our study, 2 techniques for the measurement of skin erythema were compared. Variables such as precision, agreement, and correlation are well-accepted tools for method comparison studies. The variation of repeated measurements of both techniques was < 10%. Ideally this value should be compared with repeated visual evaluation. However, the range of normal skin color was narrow, and clinical assessment only fell into the categories of no and slight erythema; therefore, this comparison was not made.

It is difficult to conclude whether a CV% below 10% is an acceptable amount of precision. The value should be related to actual measure in question. In the validation of diagnostic tests for hematologic laboratories it is commonly accepted that imprecision should be < 5%. However, in vivo evaluations are subject to variation variables with larger fluctuations; hence, a higher amount may be tolerated. The variation of repeated measurements include variation in techniques, variation in applying the instrument, and biological variation of the object. Consequently, each technique has its own amount of uncertainty and should be validated separately.

In humans, Fullerton et al. found a high repeatability using a chromameter with CV% for a* values that ranged from 1.83 to 11.92% (30 repeated measurements on the flexor surface of the forearm). Results of the same study revealed a high repeatability when measuring pink and red plates (CV% for a* values ranged from 0.1 to 0.74% and 0.04 to 0.22%, respectively). Another study investigated treated and untreated erythematous skin (CV% for a* values were 4.7 and 2.6%, respectively). These values resemble the estimates obtained in our study. Pet dogs that are not sedated can be less easy to keep still and evaluate than people or experimental animals, and it may be difficult to control variables of variation in such studies on dogs. A high reproducibility of chromameter erythema measurements has been found in humans (between observer, interinstrument, and day-to-day variation). These parameters have not been evaluated in dogs.

To our knowledge, there are no published studies of repeatability of skin erythema evaluation by use of present image analysis of digital photographs. Mattson et al. used image analysis of digitized color slides of burn injuries and found only relatively small differences of replicate photographic method.

In our study, agreement between technically derived erythema values was so that, except from a few outliers, the values all were within the 95% confidence interval. The range, however, was quite high, and one should hesitate when trying to estimate 1 value from the other.

The positive high correlation between the methods used confirmed the expected association between 2 techniques that measure erythema. The photographic method of erythema estimation has not been described.
for dog skin. Takiwaki and coworkers found a high positive correlation ($r = 0.91$) between chromametric $a^*$ values and quasi-$a^*$ values obtained from analysis of videomicroscopic images of human integument. Results of other studies using a chromameter indicate that there is a moderate-to-high correlation with visual score of skin erythema.

Regional variation in skin erythema was found with both techniques. The axillary region and groin area had high erythema values. These regions have thin skin, compared with the more dorsal parts of the body. The lateral aspect of the thorax and loin area had lower and almost equal erythema values. The main chromophores of skin are hemoglobin and melanin. In skin with a normal appearance, it is the horizontal subpapillary plexus that contributes to skin color in humans. In dogs, the plexus corresponds to the superficial layer plexus. The ventral wall has a well-defined papillary layer of the dermis and prominent capillary loops of the superficial plexus. In the axillary region the blood supply is less copious. An increased blood volume as the result of higher skin temperatures in such intertriginous areas could be a reason for increased erythema. In fact, a study of the microcirculation in dog skin has shown a regional variation in blood flow in the hindquarters and ventral aspect of the abdomen, the latter having blood flow nearly 4 times as high. Significant positive correlation between skin erythema and blood flow measurements has been found in a study in humans. Hence, differences in regional blood volumes and in skin thickness could explain some of the interregional variation of skin erythema.

Differences in hair density and skin pigmentation most likely contributed to the regional variation of erythema as well. Pigmentation was profound on the nose and the digital pad, and here the erythema values were significantly lower. In addition there was a substantial difference between the 2 methods, probably as the result of the special anatomy of this tissue and unequal light registration of the shiny nose. At this stage the techniques do not seem applicable in these regions.

Apart from nose measurements, both techniques had the same relationship between regional erythema in the 2 groups of dogs. A significant interregional difference was found in both groups, and measurements should be made in the same region if absolute values are used. However, if relative values were used, the measurements from various regions may be pooled. This, of course, depends of the actual reaction in question, which in itself may be site-specific. A high between-dog variation was found, especially among mixed-breed dogs. Results of right and left side comparisons indicated that symmetry in skin erythema is present on the lateral aspect of the thorax and loin and groin areas, and these regions could be treated together. The ambient temperature was within a narrow range, and the effect on skin erythema was considered minimal.

References


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