

Evaluation of non-invasive bioforensic techniques for determining the age of hot-iron brand burn scars in cattle

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ABSTRACT: Hot-iron branding is a traditional form of permanent cattle identification in the United States. There is a need for science-based determination of cattle brand age. Near infrared reflectance spectroscopy (NIRS) has been used to obtain information about animal tissues and healing processes. Height-width allometry and NIRS were applied to hot-iron cattle brand scars to determine if either or both of these methods can be used to non-invasively establish the interval since the application of hot-iron cattle brands. Length and width of a brand routinely applied to calves (~30–60 d old) were established and then the same measurements were recorded on 378 calfhood branded cattle of known age ranging from 0.5 to > 6.5 yr-of-age. Brand width and height increased over the original measurements by > 100% between calfhood application and 2.5 yr-of-age ($P < 0.001$). Brand size did not change dramatically between 2.5 and > 6.5 yr, however, both width and height were ($P < 0.05$) greater at maturity than at weaning. Near infrared spectra were collected from a) branded skin b) non-clipped (hair), non-branded skin, and c) hair clipped, non-branded skin on *Bos taurus* cross calves. Individual trial calibrations yielded high R^2 and low SE of

calibration values as well as similar cross validation performance ($P < 0.001$). Numerically lower but still strong performance ($P < 0.001$) resulted from combined data set calibrations. Cross-trial prediction of brand age was unsuccessful. One single year calibration underpredicted ($P < 0.001$) brand age of an independent validation set by 2.83 d, and another single year calibration underpredicted ($P < 0.001$) the same validation set by 9.91 d. When combined, these two datasets resulted in a calibration that overpredicted brand age in the validation set by 6.9 d ($P < 0.02$). Discriminant analyses for identification of skin surface type yielded success rates of 90% for branded, 99% for non-clipped, non-branded, and 96% for clipped, non-branded ($P < 0.01$). Discriminant analyses were also performed on samples grouped into a) less than 33 d, b) 141–153 d, and c) 169 d categories. All group membership identifications were successful at greater than 90% ($P < 0.01$). Preliminary results indicate that brand size could be used to indicate brand age and that NIRS can predict brand age as well as discriminate between broad brand age groups in cattle. More work will need to be done before these techniques can be used in real-world forensic applications.

Key words: bioforensic, burn scar, cattle, hot-iron brand, near infrared spectroscopy

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Transl. Anim. Sci. 2021.5:1-10
doi: 10.1093/tas/txab108

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Received March 2, 2021.

Accepted June 10, 2021.

INTRODUCTION

Hot-iron branding is a traditional form of permanent cattle identification practiced in multiple cultures dating back centuries (Khan and Mufti, 2007; Rajaram and Shelly, 2012; Stamp, 2013). In the United States, approximately half of all cattle are identified in this manner and the practice is more prevalent (~80%) in the western states (USDA, 2008). Although registered brands are considered an effective method of confirming ownership of livestock (American Association of Bovine Practitioners, 2020), theft can be facilitated by placing a brand on un-branded animals or by altering an existing brand. Unfortunately, cattle theft is not a thing of the past. White collar theft involving cattle is increasing (Mulder, 2019) but more traditional rustling continues to impact the ranching industry as well. Multiple states have reported a significant incidence of physical cattle theft in recent years (American Association of Bovine Practitioners, 2020). Law enforcement division data from the Texas and Southwestern Cattle Raisers Association (personal communication) indicate that in 2020 alone there were case reports involving 9,142 “missing” cattle. Of this number, 582 animals valued at \$650,569 were recovered. Regardless of methods employed, each of these crimes are fueled by drought effects on cattle prices, the boom-and-bust availability of petroleum industry jobs in rural areas and unfortunately, rural drug use is also a contributing factor (Montlake, 2018).

During a period of record high cattle prices (2011–2015), Arizona Department of Agriculture livestock inspectors (personal communication) reported instances of criminal charges being dropped in cases where individuals were found in possession of allegedly stolen cattle. Specifically, expert opinion indicated that the cattle in question exhibited brands appearing to have been altered from the original design or, that “calf-size” brands had been recently applied to mature cattle. In these instances, law enforcement officials did not have a readily available forensic method to determine that the alleged altered portion of the brand was “newer” than the supposed original brand, nor did they have access to scientific reference data with which to establish that a given brand had or had not grown to the size expected for mature cattle.

Hot-iron branding, achieved by flame or electric heating, causes a wound that takes ≥ 8 wk to heal in cattle (Tucker et al., 2014a). This wound ultimately results in a scar (i.e. a brand). Although it seems intuitive that a recently altered brand would

be visually identifiable or that a brand created on cattle at an early age would change and grow in size with the animal, no specific scientific documentation to that effect was found. In fact, with respect to scars in general, Bond et al. (2008) reported that there has been no formal description in the literature of how clinical characteristics of scars change with time.

Merriam-Webster defines forensic science as the application of scientific principles and techniques to matters of criminal justice especially as relating to the collection, examination, and analysis of physical evidence. Despite attribution of relative speed and infallibility with regard to forensic techniques in popular culture (i.e. “The CSI Effect”), certain analytical methods and the interpretations thereof have been called into question by the scientific community. A National Academy of Sciences report (NAS, 2009) on the subject called for research to address issues of accuracy, reliability, and validity. Murrie et al. (2019) surveyed 183 practicing forensic analysts in the United States and found that less than 7% could identify published scientific error rates for their respective techniques and that a significant number of those surveyed placed estimated error rates at unrealistically low values. In a follow up to the NAS (2009) report, Bell et al. (2018) opined that forensic science is critical to the administration of justice, but many of the techniques were developed and vetted by law enforcement and the legal system and have not been subjected to an appropriate level of scientific scrutiny. Presumption of innocence is a legal concept recognized in many countries which places the burden of proof in a criminal trial on the prosecution. There is thus a universal need for verifiable forensic techniques to help determine innocence or guilt. With respect to cattle theft, there is a specific need for field expedient methods to determine if a brand has or has not been altered, or to verify a date of application.

The healing process subsequent to any disruption of cutaneous integrity involves inflammatory (1 to 3 d), proliferative (4 to 21 d), and remodeling (22 to 365 d) phases (Profyris et al., 2012; Rowan et al., 2015; Hawkins et al., 2018). Huebner et al., (2017) and Shehata et al. (1992) describe similar processes in cattle and buffalo, respectively. Comparative healing in cattle and horses has been characterized by Dinev and Dzhurov (1987) who found that cattle exhibited greater connective tissue remodeling than horses 2–3 wk after a cutaneous tissue wound. Munro and Munro (2013) state that these processes are variable in length and physiology, a fact that

complicates the determination of wound age in a forensic analysis.

Studies conducted by Tucker et al., (2014a; 2014b) reported 67% and 46 % of hot-iron brands had healed by 7 wk post-application in Angus-Hereford calves at 4 vs. 7 mo-of-age, respectively. Scar maturation also occurs at different rates for different ages in humans (Bond et al., 2008). Human mast cell populations change in scar tissue as it ages (Hermes et al., 2000). Rawlins et al. (2006) observed changes in collagen as burn scars mature and found an increase in the Type I/Type III collagen ratio compared to normal skin. The altered ratio is evident in a transformation of collagen from a basketweave arrangement to one of the small parallel bundles. These authors report that edema affects the orientation of collagen fibers as well and may contribute to the differences observed in young and old burn wounds. During the remodeling phase, due largely to the Type I/III collagen ratio, scar tissue becomes visibly different than normal or un-injured skin. Other contributing factors include loss of hair follicles and sebaceous glands (Profyris et al., 2012). Rawlins et al. (2006) state that “...despite a great deal of research involving early burn wounds, few investigators have studied the histology of mature burn scars or burn scar contractures. To better understand how burn scars develop and mature with time, it would seem logical to study burn scars and burn scar contractures in their mature forms.” Subsequent research has since added to the body of knowledge concerning mature burn scars (Brusselaers et al., 2010; Nedelec et al., 2014; Lee et al., 2016), however, the authors agree with Rawlins et al. (2006) in principle and would add that although there is literature on burn scar healing processes, no information exists specifically to inform forensic ageing of burn scars resulting from the application of hot-iron brands to cattle.

Near infrared reflectance spectroscopy (NIRS) involves the detection of electromagnetic radiation, or light, in the near infrared (NIR) band (~800–2500 nm) that has been reflected by a substance of interest. Upon irradiation, organic bonds, primarily CH, OH, and NH, begin to vibrate at characteristic frequencies which correspond to those found within the NIR band. Similar to the perception of color in the visible band (~380–780 nm), light energy at similar wavelengths/frequencies is absorbed by a given bond, dissimilar wavelengths are reflected. This occurrence results in a biochemical “snapshot” of the material, via summary of the relative population of bonds present and their detected absorbance and reflection of NIR

light. Near infrared spectroscopy is widely used in grazing animal nutrition (Dixon and Coates, 2009) and wildlife ecology (Vance et al., 2016) as a rapid, non-invasive, non-destructive method for the determination of many biochemical constituents.

The NIRS technique has been used in medical disciplines to non-invasively monitor parameters such as blood oxygen and hemoglobin, or to discriminate between cancerous and non-cancerous tissue (Ferrari et al., 2012). Forensic scientists have also explored the technique. Brandes (2009) used portable NIRS in a forensic application to determine sex and race from human hair samples. Pringle et al. (1999) evaluated the effect of pigment in hoof, horn, and hair on NIRS analyses in sheep and horses. More germane to our discussion is that Sowa et al. (2001) evaluated burn injury hemodynamics, i.e. variation in blood flow and composition, in pigs using NIRS and found the technique to be valuable for early assessment of burn injury due to detectable changes in water, and hemoglobin oxygen saturation. This group later reported NIRS discrimination of shallow and deep porcine burn injuries (<1% total body surface area) with > 80% success (Sowa et al., 2006). Additionally, Yeong et al. (2005) found that NIRS on day 3 post-burn discriminated between burns (<20% total body surface area) that healed in less than 14 d vs. those that healed in greater than 14 d in human subjects with an overall 86% success rate.

Near infrared spectroscopy techniques would seem to be a natural fit for the forensic evaluation of the hot-iron brand burn scar healing process. Due to the need for fast, accurate, forensic methods to determine the age of hot-iron cattle brands, four trials were conducted to test the following hypotheses: a) burn scars resulting from hot-iron brands applied to cattle as a calf will grow in proportion with the animal until maturity, and b) physical and biochemical changes in the burn scar will be detectable via portable NIRS. The objectives of this research were to: a) determine the relationships between hot-iron brand age, size, and animal growth, and then use this information to develop an allometric calibration with which to estimate brand age in cattle and, b) determine the efficacy of portable NIRS as a bioforensic tool with which to predict the age of hot-iron brands as applied to cattle.

MATERIALS AND METHODS

Animal procedures were conducted at the University of Arizona’s V Bar V Ranch Agriculture Experiment Station near Camp Verde (34.6, 111.7)

or at the West Campus Agricultural Center in Tucson (32.2, 111.0) in accordance with protocols approved by the University of Arizona Institutional Animal Care and Use Committee.

Objective 1

In Trial 1, in an effort to determine the relationships between hot-iron brand age, size, and animal growth, the width (4.5 cm) and height (5.7 cm) of the upper half of the V Bar V cattle brand as illustrated in Fig. 1 were established. This brand is routinely applied to calves (~30–90-d-old) over the left ribcage with an electrically heated iron concurrently with other health procedures such as vaccination and castration in late spring. Using calipers, we then obtained the same measurements from 378 calfhood branded *B. taurus* cross cattle of known age ranging from 0.5 to > 6.5 yr-of-age during routine health and management “workings” when cattle were gathered in the fall. General linear model procedures (McCullagh and Nelder, 1989) were used to detect differences in brand measurements due to age. Mean separation was accomplished via the Tukey–Kramer test (Kuehl, 1999). Regression procedures (Steel and Torrie, 1980) were applied to determine fit of the age by width or height relationship curve. Significance was determined at $P < 0.05$.

Objective 2

In Trials 2–4, in an effort to evaluate the ability of NIRS to predict the age of hot-iron brands, visible and near infrared spectra (400–2500 nm) were collected with an ASD Field Spec Pro[®] equipped

with a fiber optic contact probe (Fig. 1) from three replicates of a) branded skin, b) non-clipped (hair), non-branded skin, and c) hair clipped, non-branded skin (Fig. 1). In Trial 2 (2014) 12 (seven male, five female) 30 ± 7 -day-old spring born calves (40 ± 5 kg) representative of the *B. taurus* cross commercial herd were utilized at the V Bar V Ranch. On the first collection date, electrically heated brands were applied to calves on the left rib cage in conjunction with routine calfhood health and management procedures. At 2 h post-branding, the hair adjacent to the brand on each calf was clipped prior to the collection of spectra. Spectra collection took approximately 1 min per animal. Other than the 2 h waiting time after brand application, spectra were subsequently collected in the same manner at 33 and 153 d-post-branding, concurrent with artificial insemination and weaning activities of the herd, respectively. Trial 3 (2015) involved 14 heifer calves at the same location, and were of similar breeding, age, and weight as those utilized in Trial 1. Spectra were collected as in Trial 1 on d 0 and 141 post-branding, i.e. at branding and weaning. In Trial 4 (2015) spectra were collected from brands that had been applied 169 d earlier to 40 weaned *B. taurus* cross steers (235 ± 10 kg) housed at the university-owned feedlot. These steers originated from the V Bar V Ranch. Spectra collection procedures were the same as those employed in Trials 2 and 3.

Chemometric Analyses

In Trials 2 to 4 which utilized spectroscopy of cattle brands, NIR spectra were averaged by animal and date of collection within skin surface type (i.e. branded skin; non-clipped (hair), non-branded



Figure 1. Left – Example of hot-iron cattle brand burn scar (applied to the left ribcage with an electrically heated iron) within approximately 1-hour post-application. Photograph includes illustration of the height and width measurements as applied in this study as well as (A) branded skin, (B) non-clipped (hair), non-branded skin, and (C) hair clipped, non-branded skin areas. Right – Illustration of near infrared spectra collection using contact probe.

skin; and hair clipped, non-branded skin). Spectra were obtained as log 1/reflectance (Workman, 2004). Prior to calibration development, spectra were subjected to a baseline correction and either a first or second derivative (Duckworth, 2004) to account for any differences in temperature, path-length, and scatter that might arise during scanning the surface of a live animal. Prediction of brand age was accomplished using partial least squares regression procedures (Westerhaus et al., 2004) due to the fact that this multivariate technique allows for modelling covariance in both spectral and reference data. Identification of skin surface type or brand age groups was achieved with linear discriminant analysis of principal component scores (Westerhaus et al., 2004) to take advantage of data compression and reduction of multicollinearity inherent in NIR spectra. In each case, cross validation (Stone, 1974) and Chi-square (Steele and Torrie, 1980) procedures were used to evaluate calibration effectiveness. Significance was determined at $P < 0.05$. Additionally, individual calibration effectiveness was ascertained by cross trial validation (e.g. Trial 2 calibration used to predict Trial 3 samples) as well as by prediction of an independent data set collected in Trial 4.

RESULTS

Objective 1

Number of animals sampled, mean and standard error values for brand measurements in each age group are presented in Table 1. Brand width and height increased over the original electric branding iron measurements by greater than 100% between calfhoo application and 2.5 yr-of-age (Table 1; Fig. 2, $P < 0.001$). Brand size did not change dramatically between 2.5 and > 6.5 yr, however, both width and height were significantly ($P < 0.05$) greater at maturity than at weaning. There was a significant quadratic relationship between brand age and both physical brand size measurements (width $r^2 = 0.20$, height $r^2 = 0.51$; $P < 0.001$).

Objective 2

Characteristics of all calibration and validation data sets are found in Table 2. Calibration and cross-validation performance results are found in Table 3. Individual trial calibrations yielded high R^2 and low SE of calibration (SEC) values as well as similar cross validation performance ($P < 0.001$).

Table 1. Effect of animal age on growth of hot-iron cattle brands originally applied to *B. taurus* cross calves on the left ribcage at approximately 30–90 days-of-age

Age	N	Width		Height	
		Mean	Std. Err.	Mean	Std. Err.
0.5	21	9.06 ^a	0.28	7.30 ^a	0.19
1.5	121	11.66 ^{b,e,f}	0.11	9.75 ^{b,e,f}	0.11
2.5	91	10.59 ^{c,e,f}	0.15	11.95 ^{c,d,e,f}	0.15
3.5	61	10.81 ^{c,d,e,f}	0.16	12.18 ^{c,d,e,f}	0.15
4.5	24	10.74 ^{b,c,d,e,f}	0.23	12.75 ^{a,c,d,e,f}	0.23
5.5	28	11.11 ^{b,c,d,e,f}	0.29	13.43 ^{a,c,d,e,f}	0.38
>6.5	31	13.11 ^g	0.40	12.05 ^g	0.42

^{a,b,c,d,e,f,g}Means within a column with different superscripts differ, $P < 0.05$.

All brands were produced by electric irons of the same design and uniform dimensions (width = 4.5 cm, height = 5.7 cm).

Numerically lower but still strong performance ($P < 0.001$) resulted from combined data set calibrations. Prediction of all brand age samples is illustrated in Fig. 3.

Cross-trial prediction of brand age was unsuccessful (data not shown). On average, R^2 values were less than 0.39 and SE of prediction were greater than 65 d. Prediction of Trial 4 samples, singularly or with combined data sets was variably successful (Fig. 4). The Trial 2 calibration underpredicted ($P < 0.001$) Trial 4 by 2.83 d and Trial 3 underpredicted ($P < 0.001$) Trial 4 by 9.91 d. Alternatively, the combination of Trials 2 and 3 combined overpredicted Trial 4 by 6.9 d ($P < 0.02$).

Discriminant analyses for identification of skin surface type yielded success rates of 90% for branded, 99% for non-clipped, non-branded, and 96% for clipped, non-branded ($P < 0.01$).

Discriminant analyses were also performed on all spectra, grouped into a) less than 33 d, b) 141–153 d, and c) 169 d categories. Percent correct group identifications from discriminant analyses are found in Table 4. All group membership identifications were successful at greater than 90% ($P < 0.01$). Representative spectra from these categories are illustrated in Fig. 5.

DISCUSSION

The finding that brand scars increased in size as the animals grew from calves to 2.5 yr-of-age is consistent with practical observations in the industry (Valdez, 2007; Thomas, 2015; Lalman et al., 2019). The results of this current study also agree with Meyer et al. (2003) in which laceration scars

on young ostriches measured at 14 mo increased in size with time since injury. These authors observed that injuries occurring at approximately 1 mo-of-age generally resulted in larger scars at slaughter than those occurring at 7–10 mo-of-age. Their observations supported a hypothesis that scars grow in synchrony with the skin, but they were unable to substantiate their findings in the literature. Observations from the study reported here support Meyers' working hypothesis. However, even though hot-iron brand burn scar growth curves in this study closely resemble those expected from overall height and body mass of beef cattle (Robbins et al., 2005; Freetly et al., 2011; Goldberg and Ravagnolo, 2015) and could be used to inform the determination of brand age if the size of the original branding iron is known; more observations with a greater variety of animals and brands in a range of production and nutritional environments will be required to develop algorithms robust enough for routine use in forensic applications. The effects of such factors as a) age at brand application, b) breed type, c) sex, d) body condition, and e) brand location

(i.e. rib vs. hip) on scar healing and growth are yet undetermined. For instance, Hedrick et al. (1967) in a review of meat animal growth and development wrote that although the shape of the overall growth curve is similar between species, inflection points occur at different stages among species and different body parts grow at different rates within a species. They further note proportional growth rates within a species are affected by age, sex, and nutrition. Although genetics and selection have certainly changed since these authors described growth of carcass components, it is not unreasonable to surmise that their general principles still hold true and that the skin covering these carcass areas would be similarly affected. Additional data collection that takes the aforementioned factors into account, will lead to refinement of allometric relationships and establishment of corresponding error rates. These further developments will address concerns expressed by the forensic science community (Bell et al., 2018).

In addition to the allometric relationships described above, results of this project demonstrate

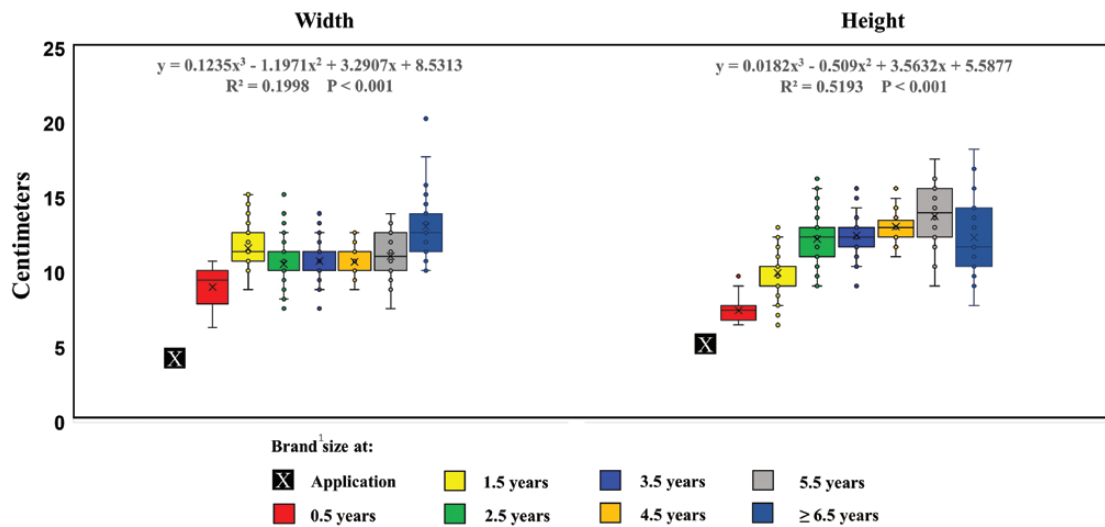


Figure 2. Effect of individual animal age on growth of hot-iron brand burn scars. Brands originally applied to *B. taurus* cross calves (~30–90-day-old) on the left ribcage with an electrically heated iron.

Table 2. Near infrared reflectance spectroscopy calibration data sets from three trials used to predict days post-application of hot-iron brands to growing *B. taurus* cross cattle

Calibration Data Set ^a	Year	Age		Number of Animals	Days Post-Application
		Class	Sex		
Trial 2	2014	Calf	M, F	12	0, 33, 153
Trial 3	2015	Calf	F	14	0, 141
Trial 4	2015	Weanling	M	40	169
Trials 2 and 3 combined	2014–15	Calf	M, F	26	0, 33, 141, 153
Trials 2, 3 and 4 combined	2014–15	Calf, Weanling	M, F	66	0, 33, 141, 153, 169

^aData sets 2 and 3 originated from the University of Arizona's V Bar V Ranch near Camp Verde, AZ. Data Set 4 originated from the University's West Campus Ag Center in Tucson, AZ.

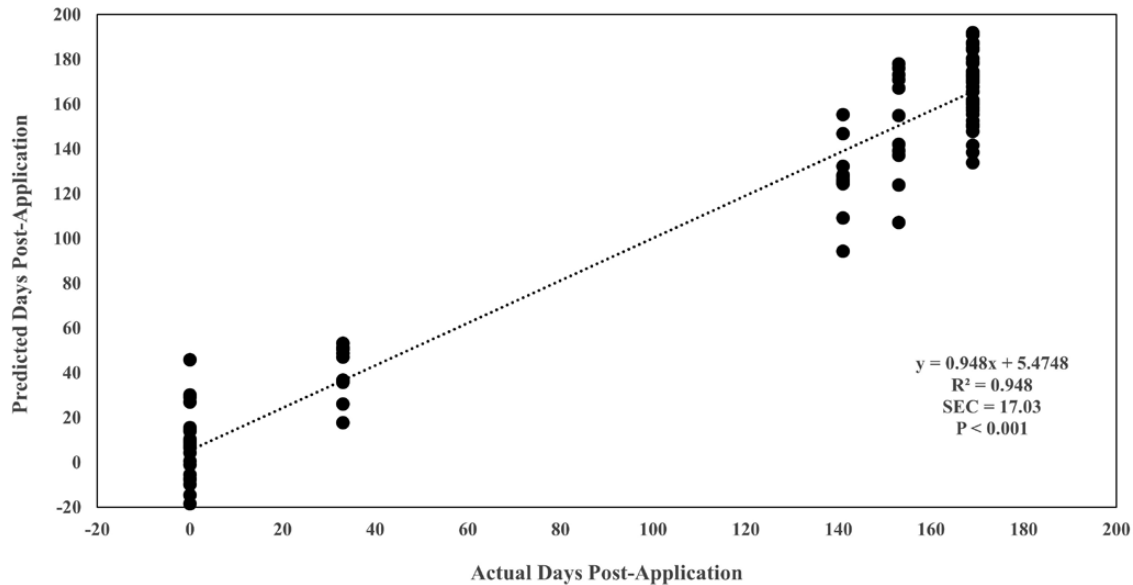
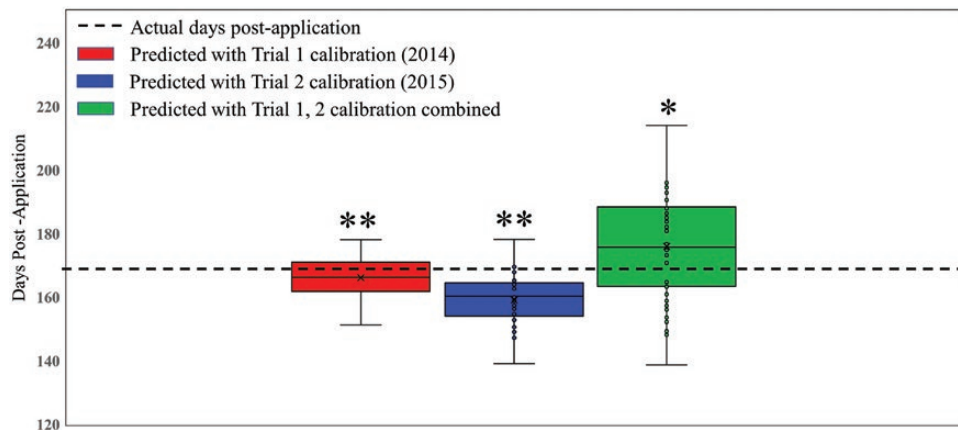
Table 3. Calibration and full cross validation results for numerical prediction of days post-application of hot-iron brands in growing *B. taurus* cross cattle

Data Set ^a	RSQ ^a	SEC ^b	P	RSQ ^a	Slope	Bias	SECV ^c	P
Trial 2	0.99	5.30	0.001	0.99	0.99	-0.46	7.46	0.001
Trial 3	0.99	6.08	0.001	0.97	0.98	-0.06	12.37	0.001
Trials 2 and 3 combined	0.92	20.42	0.001	0.90	0.91	-0.54	23.36	0.001
Trials 2, 3, and 4 combined	0.95	17.04	0.001	0.93	0.94	-0.34	19.50	0.001

^a Multiple coefficient of variation.

^b Standard error of calibration.

^c Standard error of cross validation.

**Figure 3.** Relationship between actual and near infrared spectroscopy-predicted days post hot-iron brand application in growing cattle. Calibration data set derived from three trials in 2014 and 2015 combined.**Figure 4.** Prediction of days post-application in hot-iron cattle brand burn scars. Validation of three calibrations from trials in 2014, 2015, and 2014–2015 combined. All validation spectra collected on day 169 post-application. * $P < 0.02$, ** $P < 0.001$.

for the first time that near infrared spectra from hot-iron brand burn scars are different than that of unbranded skin and that differences in branded skin change through 169 d post-application in cattle. This observation is similar to that of McIntosh et al. (2001) who reported successful NIRS discrimination between normal

skin and skin lesions, and between lesion type in humans.

This is not surprising due to the fact that the biochemical composition of burn scars differs from that of “normal” or un-burned skin. In particular, Rawlins et al. (2006) observed an increase in Type I collagen, a decrease in Type III collagen, and less

extracellular space in approximately 19-mo-old human burn scars compared to undamaged skin. They additionally note a parallel rather than basketweave arrangement of collagen fibers in burn scarred vs. normal skin, a phenomenon also reported by Zuijlen et al. (2003). Further, NIRS detected hemodynamic changes in burned vs. unburned porcine skin (Sowa et al., 2001).

The current study is a proof of concept similar to that reported by Landsman (2020) who found NIR spectral patterns associated with wound bed oxygenation and with the likelihood of closure in human diabetic or venous ulcers, but cautioned that more work is necessary. There were strong correlations observed between brand age and near infrared spectra in our study. These relationships resulted in generally successful predictions of actual brand age or correct placement into broader age groups. Standard errors associated with prediction of the independent validation set at 169 d post application indicate that accurate and precise establishment of brand age is possible. However, the poor performance observed for cross trial prediction and

the SE of cross validation within trials that ranged from plus or minus 7 to 23 d led to the conclusion that the technique of evaluating hot-iron brand age in cattle shows promise, but in agreement with the conclusion of Landsman (2020), the technique requires further refinement and validation. Therefore, with respect to establishing a date of brand application, the results presented here to establish an initial precision of approximately 2–6 wk via NIRS. Additionally, an error rate of approximately 10% could be expected when discriminating “older” vs. “newer” hot-iron brand scar tissue using this bioforensic method.

CONCLUSIONS

The research reported here emanated from a need communicated by livestock law enforcement officials for a scientifically sound bioforensic method to determine the age of hot-iron brands on cattle. The stated hypotheses regarding age-related physical and biochemical changes in hot-iron brand burn scars were both confirmed. The first objective of this research was to determine the relationships between hot-iron brand age, size, and animal growth, and then use this information to develop an allometric calibration with which to estimate brand age. These preliminary results indicate that if the original brand size is known, current size can be used to indicate brand age. However, the authors recommend that prior to application in real-world settings, that a more comprehensive calibration dataset will need to be collected; one that incorporates the expected range of variation in such factors as age at brand application, breed type, sex, body condition, and brand location on the body. Once

Table 4. Discrimination of brand age groups representing three different days-post-application of hot-iron brands in growing *B. taurus* cross cattle via near infrared reflectance spectroscopy. Spectral data from all trials combined

Observed Group	Percent Correct Group Identification				X^2	P
	< 33d	141–153d	169d			
<33d	94	3	3	17.7	0.0001	
141–153d	0	95	5	11.5	0.0007	
169d	3	0	97	23.3	0.0001	

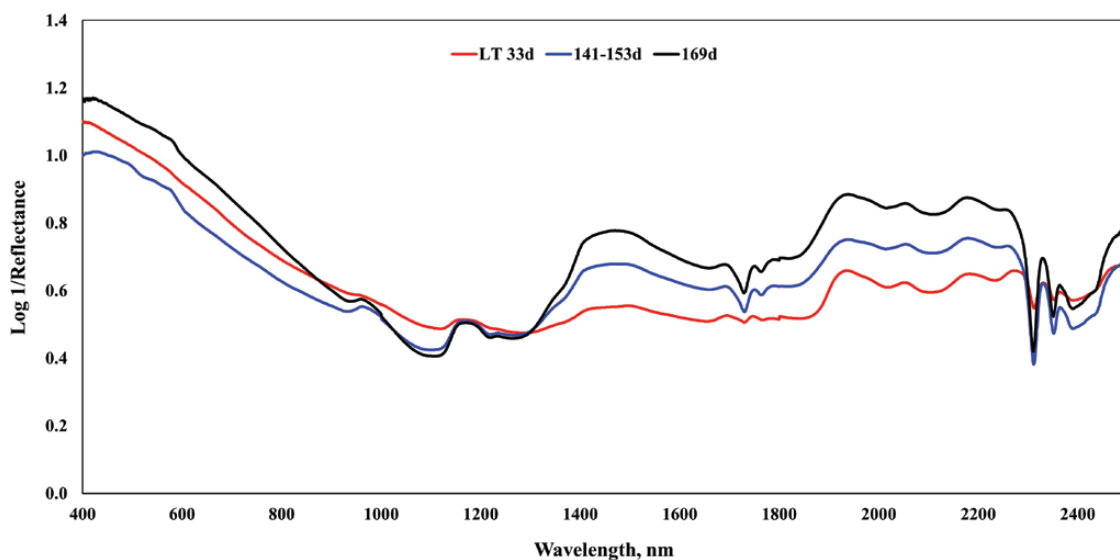


Figure 5. Near infrared absorbance spectra of hot-iron cattle brand burn scars: three different days-post-application categories.

accomplished and subjected to appropriate (and ongoing) peer review, the resultant allometric relationships would provide a field expedient method with established accuracy and error rates as called for by the forensic science profession.

The second objective of this research was to determine the efficacy of portable NIRS as a bioforensic tool with which to predict the age of hot-iron brands as applied to cattle. Again, these preliminary results indicate that NIRS performed with portable equipment on a live animal in the field can predict brand age, as well as discriminate between broad brand age groups. This is the first report of NIRS used on live subjects for a forensic purpose. As stated above, similar cautions with respect to further refinement of the technique prior to adoption are recommended.

This report provides a starting point for the development of practical bioforensic tools to be used in determining the age of burn scars. Such tools could be applied to humans as well as livestock.

ACKNOWLEDGMENTS

The authors would like to acknowledge the contributions to this study made by Keith G. Cannon, Keith O. Cannon, and Debra Pearson at the V Bar V Ranch, as well as Victoria Lowe at the University of Arizona Feedlot.

Conflict of interest statement. None declared.

LITERATURE CITED

- American Association of Bovine Practitioners. 2020. Summary: AABP animal welfare committee branding working group activities. 4 pp.
- Bell, S., S. Sah, T. D. Albright, S. J. Gates, Jr, M. B. Denton, and A. Casadevall. 2018. A call for more science in forensic science. *Proc. Natl. Acad. Sci. U. S. A.* 115:4541–4544. doi:10.1073/pnas.1712161115.
- Bond, J. S., J. A. L. Duncan, A. Sattar, A. Boanas, T. Mason, S. O’Kane, and M. W. J. Ferguson. 2008. Maturation of the human scar: an observational study. *Plast. Reconstr. Surg.* 121:1650–1658. doi:10.1097/PRS.0b013e31816a9f6f.
- Brandes, S. 2009. Near-infrared spectroscopic studies of human scalp hair in a forensic context [M. App. Sc. Thesis]. Brisbane City, Queensland, Australia: Queensland University of Technology. p. 231.
- Brusselaers, N., A. Pirayesh, H. Hoeksema, J. Verbelen, S. Blot, and S. Monstrey. 2010. Burn scar assessment: a systematic review of different scar scales. *J. Surg. Res.* 164:e115–e123. doi:10.1016/j.jss.2010.05.056.
- Dinev, D., and A. Dzurov. 1987. Histogenesis of granulation tissue in wound healing by second intention in horses and cattle. *Vet. Med. Nauki.* 24:72–79.
- Dixon, R., and D. Coates. 2009. Near infrared spectroscopy of faeces to evaluate the nutrition and physiology of herbivores. *J. Near Infrared Spectrosc.* 17:1–31. doi:10.1255/jnirs.822
- Duckworth, J. 2004. Mathematical data preprocessing. In: C. A. Roberts, J. Workman, and J. B. Reeves, editors, *Near-infrared spectroscopy in agriculture* (Vol. 44). American Society of Agronomy, Madison, WI. p. 115–132.
- Ferrari, M., K. H. Norris, and M. G. Sowa. 2012. Guest editorial: medical near infrared spectroscopy 35 years after the discovery. *J. Near Infrared Spectrosc.* 20:vii–ix. doi:10.1255/jnirs.982
- Freetly, H. C., L. A. Kuehn, and L. V. Cundiff. 2011. Growth curves of crossbred cows sired by Hereford, Angus, Belgian Blue, Brahman, Boran, and Tuli bulls, and the fraction of mature body weight and height at puberty. *J. Anim. Sci.* 89:2373–2379. doi:10.2527/jas.2011-3847.
- Goldberg, V., and O. Ravagnolo. 2015. Description of the growth curve for Angus pasture-fed cows under extensive systems. *J. Anim. Sci.* 93:4285–4290. doi:10.2527/jas.2015-9208.
- Hawkins, H. K., J. Jayson, and C. C. Finnerty. 2018. Pathophysiology of the burn scar. In: D. N. Herndon, editor, *Total burn care*. 5th ed. New York, NY: Elsevier. Inc; p. 466–475. doi:10.1016/B978-0-323-47661-4.00044-7
- Hedrick, H. B., D. W. Zinn, S. F. Zobrisky, and M. F. Bailey. 1967. Quantitative relationships of meat animal composition during growth and development. In: *Proc. Reciprocal Meat Conf.* p. 55–87.
- Hermes, B., I. Feldmann-Böddeker, P. Welker, B. Algermissen, M. U. Steckelings, J. Grabbe, and B. M. Henz. 2000. Altered expression of mast cell chymase and tryptase and of c-Kit in human cutaneous scar tissue. *J. Invest. Dermatol.* 114:51–55. doi:10.1046/j.1523-1747.2000.00837.x.
- Huebner, K. L., A. K. Kunkel, C. S. McConnel, R. J. Callan, R. P. Dinsmore, and L. S. Caixeta. 2017. Evaluation of horn bud wound healing following cautery disbudding of preweaned dairy calves treated with aluminum-based aerosol bandage. *J. Dairy Sci.* 100:3922–3929. doi:10.3168/jds.2016-12192.
- Khan, S., and O. Mufti. 2007. The hot history and cold future of brands. *J. Manage. Sci.* 1:75–87. http://www.qurtuba.edu.pk/jms/default_files/JMS/1_1/5_saif_ullah.pdf
- Kuehl, R. O. 1999. *Design of experiments: statistical principles of research design and analysis*. 2nd ed. Pacific Grove, CA: Duxbury Press.
- Lalman, D., C. Danielson, and M. Kelsey. 2019. Livestock branding in Oklahoma. Stillwater, OK: Oklahoma Cooperative Extension Service AFS-3255-4.
- Landsman, A. 2020. Visualization of wound healing progression with near infrared spectroscopy: a retrospective study. *Wounds* 32:265–271.
- Lee, K. C., J. Dretzke, L. Grover, A. Logan, and N. Moiemien. 2016. A systematic review of objective burn scar measurements. *Burns Trauma* 4:14. doi:10.1186/s41038-016-0036-x.
- McCullagh, P., and J. A. Nelder. 1989. *Generalized linear models*. 2nd ed. London: Chapman and Hall.
- McIntosh, L. M., R. Summers, M. Jackson, H. H. Mantsch, J. R. Mansfield, M. Howlett, A. N. Crowson, and J. W. Toole. 2001. Towards non-invasive screening of skin lesions by near-infrared spectroscopy. *J. Invest. Dermatol.* 116:175–181. doi:10.1046/j.1523-1747.2001.00212.x.
- Meyer, A., S. W. P. Cloete, C. R. Brown, and S. J. van Schalkwyk. 2003. The persistence to slaughter age of scars resulting from damage inflicted to ostrich skins during the grow-out phase. *S. Afr. J. Anim. Sci.* 33:32–37. doi:10.4314/sajas.v33i1.3735

- Montlake, S. 2018. The lawman and the outlaw: how cattle rustling and drugs are roiling rural America. *Christian Science Monitor*. [accessed 11-23-20] Available from <https://www.csmonitor.com/USA/Society/2018/0130/The-lawman-and-the-outlaw-How-cattle-rustling-and-drugs-are-roiling-rural-America>
- Mulder, B. 2019. In Texas, cattle rustling evolves into sophisticated multimillion-dollar crimes. *Dallas Morning News*. Available from <https://www.dallasnews.com/business/2019/05/12/in-texas-cattle-rustling-evolves-into-sophisticated-multimillion-dollar-crimes/> accessed 11-23-20
- Munro, R., and H. M. Munro. 2013. Some challenges in forensic veterinary pathology: a review. *J. Comp. Pathol.* 149:57–73. doi:10.1016/j.jcpa.2012.10.001.
- Murrie, D. C., B. O. Gardner, S. Kelley, and I. E. Dror. 2019. Perceptions and estimates of error rates in forensic science: a survey of forensic analysts. *Forensic Sci. Int.* 302:109887. doi:10.1016/j.forsciint.2019.109887.
- National Academy of Sciences. 2009. Strengthening forensic science in the United States: a path forward. *Natl. Acad. Press*, Washington, DC.
- Nedelec, B., J. A. Correa, A. de Oliveira, L. LaSalle, and I. Perrault. 2014. Longitudinal burn scar quantification. *Burns* 40:1504–1512. doi:10.1016/j.burns.2014.03.002.
- Pringle, J., C. Roberts, M. Kohl, and P. Lekeux. 1999. Near infrared spectroscopy in large animals: optical path-length and influence of hair covering and epidermal pigmentation. *Vet. J.* 158:48–52. doi:10.1053/tvjl.1998.0306
- Profyris, C., C. Tziotziou, and I. Do Vale. 2012. Cutaneous scarring: pathophysiology, molecular mechanisms, and scar reduction therapeutics. Part I. The molecular basis of scar formation. *J. Am. Acad. Dermatol.* 66:1–10. doi:10.1016/j.jaad.2011.05.055
- Rajaram, S., and C. S. Shelly. 2012. History of branding. *Int. J. Soc. Sci. Interdiscipl. Res.* 1:100–104.
- Rawlins, J. M., W. L. Lam, R. O. Karoo, I. L. Naylor, and D. T. Sharpe. 2006. Quantifying collagen type in mature burn scars: a novel approach using histology and digital image analysis. *J. Burn Care Res.* 27:60–65. doi:10.1097/01.bcr.0000192266.14329.7b.
- Robbins, K. R., I. Misztal, and J. K. Bertrand. 2005. Joint longitudinal modeling of age of dam and age of animal for growth traits in beef cattle. *J. Anim. Sci.* 83:2736–2742. doi:10.2527/2005.83122736x.
- Rowan, M. P., L. C. Cancio, E. A. Elster, D. M. Burmeister, L. F. Rose, S. Natesan, R. K. Chan, R. J. Christy, and K. K. Chung. 2015. Burn wound healing and treatment: review and advancements. *Crit. Care* 19:243. doi:10.1186/s13054-015-0961-2.
- Shehata, A., S. R. Ali, T. A. Ibrahim, and M. A. Seleim. 1992. A trial to determine age of wound after plastic mark application in cows' and buffaloes' ears. *Assiut Veterinary Medical Journal (Egypt)*. 27:223–237.
- Sowa, M. G., L. Leonardi, J. R. Payette, K. M. Cross, M. Gomez, and J. S. Fish. 2006. Classification of burn injuries using near-infrared spectroscopy. *J. Biomed. Opt.* 11:054002. doi:10.1117/1.2362722.
- Sowa, M. G., L. Leonardi, J. R. Payette, J. S. Fish, and H. H. Mantsch. 2001. Near infrared spectroscopic assessment of hemodynamic changes in the early post-burn period. *Burns* 27:241–249. doi:10.1016/s0305-4179(00)00111-x.
- Stamp, J. 2013. Decoding the range: the secret language of cattle branding. *smithsonian.com* <https://www.smithsonianmag.com/arts-culture/decoding-the-range-the-secret-language-of-cattle-branding-45246620/> accessed 10-15-19
- Steel, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics. New York, NY: McGraw-Hill Book Co.
- Stone, M. 1974. Cross-validated choice and assessment of statistical predictions. *J. Royal Stat. Soc. Series B* 36:111–147. doi:10.1111/j.2517-6161.1974.tb00994.x
- Thomas, H. S. 2015. Strike while the branding iron is hot: tips for branding livestock. *The Fence Post*. [accessed 1-11-21]. Available from <https://www.thefencepost.com/news/strike-while-the-branding-iron-is-hot-tips-for-branding-livestock/#:~:text=Larger%20brands%20with%20more%20figures,than%20a%20small%20simple%20brand.&text=If%20you%20leave%20it%20too,brand%20grows%20with%20the%20animal.%E2%80%9D>
- Tucker, C. B., E. M. Mintline, J. Banuelos, K. A. Walker, B. Hoar, D. Drake, and D. M. Weary. 2014a. Effect of a cooling gel on pain sensitivity and healing of hot-iron cattle brands. *J. Anim. Sci.* 92:5666–5673. doi:10.2527/jas.2014-7860.
- Tucker, C. B., E. M. Mintline, J. Banuelos, K. A. Walker, B. Hoar, A. Varga, D. Drake, and D. M. Weary. 2014b. Pain sensitivity and healing of hot-iron cattle brands. *J. Anim. Sci.* 92:5674–5682. doi:10.2527/jas.2014-7887.
- USDA. 2008. Beef 2007–08. Part I: reference of beef cow-calf management practices in the United States, 2007–08. Fort Collins, CO: USDA-Animal Plant Health Inspection Service-National Animal Health Monitoring System-Veterinary Services, Centers for Epidemiology and Animal Health.
- Valdez, A. 2007. How to brand the herd. *Texas Monthly*. [accessed 1-11-21]. Available from <https://www.texasmonthly.com/the-culture/how-to-brand-the-herd/>
- Vance, C. K., D. R. Tolleson, K. Kinoshita, J. Rodriguez, and W. J. Foley. 2016. Review: near infrared spectroscopy in wildlife and biodiversity. *J. Near Infrared Spectrosc.* 24:1–25.
- Westerhaus, M., J. Workman, J. B. Reeves, and H. Mark. 2004. Chapter 7. quantitative analysis. In: C. A. Roberts, J. Workman, and J. B. Reeves, editors, *Near-infrared spectroscopy in agriculture (Vol. 44)*. Madison, WI: American Society of Agronomy; p. 133–174.
- Workman, J. 2004. Chapter 2. Near-Infrared Spectrophotometers. In: C. A. Roberts, J. Workman, and J. B. Reeves, editors, *Near-infrared spectroscopy in agriculture (Vol. 44)*. American Society of Agronomy, Madison, WI, p. 11–32.
- Yeong, E. K., T. C. Hsiao, H. K. Chiang, and C. W. Lin. 2005. Prediction of burn healing time using artificial neural networks and reflectance spectrometer. *Burns* 31:415–420. doi:10.1016/j.burns.2004.12.003.
- Zuijlen, P. V., J. J. Ruurda, H. A. van Veen, J. van Marle, A. J. van Trier, F. Groenevelt, R. W. Kreis, and E. Middelkoop. 2003. Collagen morphology in human skin and scar tissue: no adaptations in response to mechanical loading at joints. *Burns* 29:423–431. doi:10.1016/s0305-4179(03)00052-4.