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14. ABSTRACT The purpose of the current research is to determine whether the spray-on application of allogeneic keratinocytes in suspension will improve epidermal wound healing of vesicating burns induced by the chemical warfare agent sulfur mustard (HD). A beige SCID mouse model is used for these experiments which are being carried out in two phases. The first phase is dose ranging. The second phase tests the efficacy of spray keratinocytes (Universal Donor) at healing HD injuries. To limit combined injuries previously observed with HD in methylene chloride, dose ranging was carried out using ethanol as diluent and HD delivered to the dorsum of depilated mice within an 8 mm diameter cloning ring. The minimal HD exposure required to generate confluent epidermal and follicular necrosis, thrombi, and inflammatory infiltrate was identified (80 µg HD in 25 µL ethanol). Both Universal Donor cells and the SF parent cells promoted healing of debrided HD wounds; engraftment was variable.					
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Introduction

We had previously developed a cell based approach for treating acute cutaneous injuries and blistering disorders classified as epidermolysis bullosa (EB; Lin and Carter, 1992). The acute injury considered for therapeutic intervention is the result of exposure to sulfur mustard (bis-(2-chloroethyl) sulfide; HD), a chemical warfare agent that alkylates DNA, RNA and proteins. Prolonged cutaneous exposure to this agent results in vesication wherein small vesicles coalesce into large blisters that can take months to heal and require prolonged hospitalization (Papirmeister et al, 1991; Graham et al., 2005). Aggressive debridement of HD injuries that remove damaged cells and alkylated extracellular matrix ameliorates the sequelae and allows utilization of cell based therapies that either provide replacement tissue or promote healing with non-damaged host derived cells (Graham et al., 2005, 2006).

Under conditions of mass casualty or extensive injury (>40% body surface) availability of an off-the-shelf product which engrafts for prolonged times may be useful. In order to offer such a product, we had genetically engineered keratinocytes that were resistant to lymphocyte mediated cytotoxicity and which failed to stimulate proliferation of allogeneic lymphocytes even after growth with interferon- γ (Tafrov et al, 2004; Gao et al., 2006). This strain was produced by insertion of three sequences: shRNA against $\beta 2$ microglobulin, antisense against the invariant chain (Ii) of MHC class II, and viral IL10 (Tafrov et al, 2004). Suppression of $\beta 2$ microglobulin and the invariant chain (Ii) of MHC class II was used to decrease antigen presentation. Viral IL10 was used to limit susceptibility to natural killer cells and to suppress production of proinflammatory mediators (Go et al, 1990; Vieir et al, 1991; Moore et al, 2001; Rousset et al, 1992). The strain of keratinocytes is the prototype of what we have termed the "Universal Donor" (UD) and was derived from normal human epidermal keratinocytes (strain SF). Both UD and SF keratinocytes can engraft onto a full-thickness wound made on beige SCID mice and generate a fully differentiated neo epidermis (Gao et al, 2006)

The purpose of the current research is to determine whether the spray-on application of allogeneic keratinocytes in suspension will improve epidermal wound healing of vesicating burns induced by the chemical warfare agent sulfur mustard (bis (2-chloroethyl) sulfide; HD). The experiment is being conducted in two Phases. Phase I is a dose ranging study to determine the dose regimen needed to induce a deep dermal/full thickness wound. We previously found that with this model methylene chloride exposure results in cutaneous injuries and therefore evaluated the usefulness of ethanol as diluent for HD. Phase II examines the efficacy of spray keratinocytes at healing HD injuries. Both SF and UD cells will be used to treat HD injuries made on beige SCID mice with or without humanized immune systems.

Body

1. Evaluation of ethanol as the diluent of choice for HD

In the previous contract period we had noted the use of methylene chloride in our model system generates cutaneous injury independent of HD. Therefore, evaluation of ethanol as a solvent was carried out. Treatments were applied within cloning rings whose rims were coated with Krytox® (Dupont, Wilmington, DE) to prevent leakage. . All animals were kept for 24hr in the Class II B3 hood in the HD facility after which they were euthanized and skin samples taken for histopathology and immunohistochemistry. Comparison of the cutaneous effects of methylene chloride and ethanol were first evaluated in beige SCID mice treated with different volumes of each solvent. The right side of the animal received methylene chloride and the left side of the animal received ethanol. As expected, less time was required for evaporation of methylene chloride. The

evaporation times of 20 μ L of methylene chloride and ethanol were 1.03 ± 0.14 min (n=5) and 4.95 ± 1.33 min (n=6), respectively, and the evaporation times of 50 μ L of methylene chloride and ethanol were 4.75 ± 0.56 min (n = 5) and > 18 min (n=2), respectively. Because 20 μ L of either solvent did not clearly create an even surface within the cloning ring and because the ethanol evaporation rates were too slow to use 50 μ L, 25 μ L of ethanol was tested. This volume of ethanol evaporated in 14.95 ± 4.95 min (n=2) and was chosen for use in future experiments. At 24-hours post treatment, animals were euthanized and skin samples fixed and embedded for histopathology. As expected, exposure to methylene chloride initiated epidermal necrosis. In the four out of five animals exposed to 20 μ L, patchy epidermal necrosis was observed, and in five out of five animals exposed to 50 μ L, epidermal necrosis was contiguous and complete (see [Appendix 2-Table 1](#)). No epidermal necrosis was observed in any of the regions treated with ethanol (20 – 50 μ L).

2. Dose ranging with ethanol as diluent

Animals were treated with 25 μ L of ethanol, or with 25 μ L of 3.2 mg/mL HD, 1.6 mg/mL HD, or 0.8 mg/mL HD in ethanol (80 μ g, 40 μ g, and 20 μ g of HD); two animals were dosed with 25 μ L of 6.5 mg/mL HD (237.5 μ g HD). After 24-hours animals were euthanized, and treatment areas excised, fixed and embedded for histology (see [Appendix 2-Table 2](#); [Appendix 3-Figure 1](#)). In animals treated with 0.8 mg/mL HD, treatment areas showed patchy epidermal necrosis without inflammatory infiltrate. In animals treated with 1.6 mg/mL HD, 3/5 treatment areas showed inflammatory infiltrate. Epidermal necrosis was contiguous in 2 of these wounds and patchy in the treatment areas of the remaining 3 animals. However, in the animals receiving 3.2 mg/mL HD epidermal necrosis and inflammatory infiltrate was observed in all treatment areas (4/4). The tissues exposed to 237 μ g of HD showed similar epidermal necrosis and inflammatory infiltrate. Therefore, 25 μ L of 3.2 mg/mL HD (80 μ g) was chosen for future treatments. In some animals tissue breakdown was found ([Figures 1d, 2, 3](#)), but no correlation with cell engraftment was detected.

Ethanol exposure did not result in adverse cutaneous effects. Inflammatory infiltrates and epidermal necrosis were limited to the HD treatment groups - consistent with previous reports in mouse and human model systems which document HD driven release of inflammatory cytokines and promotion of apoptosis (Ricketts et al, 2000; Arroyo et al, 2000, 1999; Ruff and Dillman, 2007; Rikimaru et al, 1991).

3. Evaluation of keratinocyte spray technology for sulfur mustard treated wounds

Three groups of ten animals were used in the first experiment. Half of each group was exposed to 25 μ L ethanol (control treatment) and half was exposed to 25 μ L ethanol containing 3.2 mg/mL HD. After 24-hours, group 1 was euthanized and treatment areas excised and fixed for histology, group 2 was anesthetized and exposed area excised. After placement of the cloning chamber, cell slurries of keratinocytes (strain SF transduced with empty vector) and fibroblasts (Clonetics strain, transduced with empty vector) were added (Wang et al, 2000). Group 3 animals were processed similarly but using UD keratinocytes and fibroblasts (Clonetics strain transduced with vector used to generate UD keratinocytes and expressing vIL-10). Human fibroblasts were used to mimic the cell therapy that might be brought into human use.

All areas exposed to HD showed epidermal necrosis and inflammation with varying degrees of thrombosis (see [Appendix 2 -Table 3](#)). No lesions were observed in the ethanol exposed skin. Two-thirds of the ethanol control wounds treated either with SF (mouse 2 and 11) or UD (mouse 21 and 25) cells healed with human epidermis detected at one-month post cell application (See [Appendix 2 -Table 4](#) and [Appendix 4 - Report R16948-08](#)). Only 1/5 HD exposed regions (mouse

19) that were excised and treated with the SF cell slurries retained human epidermis at one-month post cell application. This compares to the result obtain with the UD cell slurries in which 5/5 HD exposed sites (mouse 26-30) retained human epidermis. Mouse 23, which was given no cells, showed hyperplastic mouse epidermis with exuberant inflammation. No staining with anti-human Involucrin antibody was seen in the normal or hyperplastic mouse epidermis supporting the use of the anti-human involucrin antibody to identify human epidermis. However, cross-reacting material was detected in the granuloma. Therefore, unequivocal identification of the origin of cellular material within the granulomas cannot be made.

In our earlier studies, both SF and UD cells supported development and maintenance of human epidermis in this model system. Therefore, the experiment was repeated. As positive control, an additional group was treated with neonatal keratinocytes. These cells have high colony forming efficiencies and have been found by multiple laboratories to readily engraft. Shown in [Appendix 2 - Table 5](#) are the evaluations of tissues exposed to either 25 μ L ethanol or 25 μ L of 3.2 mg/mL HD in ethanol. As previously found, ethanol treated sites were without injury with the exception of three animals showing focal epidermal necrosis in < 4% of the area evaluated (possibly the result of scratching). The HD treated sites again extensive epidermal necrosis (15/15 animals) with pustule formation (14/15 animals), thrombosis (9/15 animals), and inflammation (10/15 animals). The results of cell slurry application evaluated 6-weeks post application are given in [Appendix 2 -Table 6](#) and [Appendix 4 - Report 1299-09](#). Every wound treated with the neonatal cells maintained human epidermis. However, only three treatments with the adult cells resulted in human epidermal maintenance (animal 19 – HD exposed, UD treated; animal 11 – ethanol exposed, SF treated, and animal 27 – HD exposed, SF treated).

To enhance engraftment using UD and SF cells three modifications were made: [1] 40-50% confluent cultures were used to maximize the fraction of proliferative cells, [2] cell slurry application was completed within 30-minutes of cell harvest rather than allowing a 1-hour window, and [3] the lower part of the cloning chamber was allowed to remain in place for 2-weeks rather than one week to limit re-epithelialization by mouse epidermis. These experiments are in progress.

4. Evaluation of plasma/fibrin gel to enhance keratinocyte engraftment in the mouse model

Experiments were also conducted to determine whether deposition of human keratinocytes on a human fibroblast containing plasma (high fibrin, low fibronectin concentration) gel would promote human keratinocyte engraftment. For this probe experiment surgical wounds without HD treatment were used. As control, UD and SF control cells added as cell slurries were also tested. Human fibroblasts were genetically engineered using the same retroviral vectors used to generate UD keratinocytes; control fibroblasts were transduced with empty vector. Forty percent (6/15) of animals used for these experiments died post-anesthesia. Human cells were not found in the neo-epidermis. Granulomas found around each of the wound areas are consistent with local irritation. (See [Appendix 2 -Table 7](#) and [Appendix 4 - Report 8083-09](#)).

Because the number of adverse events in this experiment could confound interpretation of results, the experiment was repeated using four groups of 5 animals each subjected to full thickness surgical wounds. UD keratinocytes and fibroblasts were added as cell slurries to two groups, one for evaluation at 4-weeks and one for evaluation at 6-weeks post application. The other two groups of mice received UD keratinocytes and fibroblasts as plasma gel constructs with one group used for evaluation at 4-weeks post application and the other group for evaluation at 6-weeks. The bottom of each cloning chamber was left in place for 2 weeks to limit re-epithelialization from the wound edge ([Appendix 3 - Figure 4](#)). Histology and immunohistochemical analyses for human Involucrin are in progress.

KEY RESEARCH ACCOMPLISHMENTS

- Completed the Phase I dose ranging using HD diluted in ethanol to avoid the combined injury observed using methylene chloride in this model system
- Initiated Phase II and demonstrated wound healing with cell slurries of UD and SF cells but variability in the maintenance of human epidermis.

REPORTABLE OUTCOMES

N/A

CONCLUSIONS

- Dose ranging on mouse dorsal skin with XHD in ethanol was completed. Using 25 μ L HD in ethanol, gave consistent epidermal necrosis with follicular involvement, inflammation, and thrombosis apparent 24-hr after dosing. No damage was observed histologically in the ethanol treated controls.
- Cell slurries of the genetically modified UD (Universal Donor) cells promoted the healing of mouse dorsal skin subjected to XHD (or ethanol as control) and debrided 24-hr post-exposure. Experiments using cells genetically modified with empty vector gave similar results.
- Although results were variable, slurries of UD cells with human fibroblasts genetically modified with the same vectors generated a neo-epidermis and dermis having significant regions of human cell engraftment.
- Engraftment frequencies unexpectedly varied between experiments necessitating further evaluations using cells harvested at lower degrees of confluence.

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APPENDIX 1. METHODS

Cell Culture

Keratinocytes (strain SF, strain UD, neonatal foreskin) were cultured with lethally-irradiated 3T3 (Rheinwald and Green, 1975) using media modifications as previously described (Randolph and Simon, 1993). 3T3 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) with 10% bovine calf serum (HyClone, Logan, Utah) and fibroblasts were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (HyClone, Logan, Utah). For experiments cultures were harvested at 60% confluence. Irradiated 3T3 feeder cells were removed using calcium/magnesium free phosphate buffered saline (PBS) with 1 mM EDTA, and keratinocytes then recovered using 5-10 min 37°C incubations with PBS containing 0.1% Trypsin and 1 mM EDTA. All keratinocyte strains used were obtained under IRB approvals which are renewed yearly. Fibroblasts (Clonetics) were transduced with empty vector or with vector containing sequences to generate UD fibroblasts using techniques described previously (TCN05077 from Battelle contract) with supplemental funding obtained from NYSTEM.

Animal Studies

The day prior to dose ranging the dorsum of each animal (Beige SCID: C.B.-17.B6-Prkdc^{scid}Lyst^{bg}/CRL, 7 weeks old; 20-24 grams) was shaved and depilated with Nair at the SBU animal facility; animals were lightly anesthetized with 2% isoflurane for restraint. At that time each animal was numbered on the tail with an indelible marking pen. On the day of dose ranging the animals were placed in individual compartments of a cage that held 4 x 3 animals. Animals were then transported to the HD facility. Animals were anesthetized with IP injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) and placed into the biosafety cabinet. Dr. John S. Graham and later Dr. Edward D. Clarkson with the aid of Dr. M. Simon then dosed the right dorsal surface with either methylene chloride, ethanol, or ethanolic solutions of HD The treatment was added within a cloning ring whose rim was coated with Krytox (Dupont, GPL203, Lot# G1330) to prevent the leaking of solvent. Cloning rings were held in place for 5-30 minutes to ensure complete evaporation and uptake of the treatment. Each animal was then placed back into its cage. For Phase I dose ranging experiments, at 24-hours post-exposure animals were euthanized with by IP injection of pentobarbital (150 mg/kg). Eight mm punch biopsies were taken of the treatment area placed between two thin sponges in a cassette and fixed in formalin.

Application of ethanol or XHD in ethanol



For Phase II experiments evaluating wound healing and potential human cell engraftment, at 24-hour post-exposure animals were again anesthetized with ketamine/xylazine (see above), wounds were debrided and cloning chambers inserted into the wound. Cell slurries were then added to the chambers. After 1 week (longer for indicated experiments), the cloning chambers were removed and wounds were allowed to heal with exposure to the air. At 4-6 weeks, animals were euthanized and the pelts containing the healed wounds were removed, fixed and evaluated histologically and immunohistochemically. Animal handling, anesthesia and euthanasia were carried out under the direction of Dr. Thomas Zimmerman, D.V.M. and Director of the Stony Brook University Division of Laboratory Animal Research.

Cloning chambers for cell application



Cells are added to opening in chamber (arrows)

Histology and Immunohistochemistry:

Biopsies were formalin fixed in a 1:4 dilution of 10% formalin in Ca-Mg free phosphate-buffered saline for 24 hours, placed in 70% ethanol and sent to McClain Laboratories, Smithtown, NY for paraffin embedding, staining and histopathology. Using standard procedures 6 µm sections were cut and deparaffinized with xylene and graded alcohols. Histopathology was carried out using H&E [Mayers hematoxylin (PolyScientific, S2697, Lot 04770) and eosin (PolyScientific, S176, Lot 03446) staining] Immunohistochemistry was carried out with antibody against human involucrin (prepared by M. Simon) using a dilution of 1:1000. Embedded tissue was placed in Blue Ribbon tissue Infiltration Medium (Surgipath), sectioned at 5 µm and baked for 1-hour at 70oc. After slides were deparaffinized and rehydrated with dH2O, slides were placed in the Trilogy Antigen Retrieval solution (Cell Marque) for 1-hour, 92°C, cooled to RT and placed in Tris Buffered Saline and Tween 20 (Labvision). Immunohistochemistry was then carried out with solutions from Biocare as follows:

- | | | | |
|----|-----------|-----------------|------------------------------------|
| a. | 10-minute | incubation with | Sniper Protein Block |
| b. | 2-hour | incubation with | primary antibody (1:1000 dilution) |
| c. | 20-minute | incubation with | Mach 4 Universal Probe |
| d. | 20-minute | incubation with | Mach 4 Universal Polymer |
| e. | 20-minute | incubation with | Vulcan Fast Red |

Sections were then counterstained with hematoxylin, and then dehydrated and coverslipped with Acrymount (Anapath) using standard procedures.

APPENDIX 2. SUMMARY TABLES

Table 1. Cutaneous effects of methylene chloride and ethanol

8/06/2008 Treatment - Histology read 8/22/2008

Animal Number	Methylene chloride (right flank)		Ethanol (left side)	
	μL	epidermal necrosis (mm) (average of two sections)	μL	epidermal necrosis (mm) (average of two sections)
1	20	0	20	0
2	20	patchy (0.1-1.9 mm lesions)	20	0
3	20	0.9 \pm 0.6	20	0
4	20	4.2 \pm 1.1	20	0
5	20	1.5 \pm 0.9	20	0
6	50	6.0 \pm 0.2	50	0
7	50	6.0 \pm 0.1	50	0
8	50	5.2 \pm 0.1	25	0
9	50	5.0 \pm 0.6	25	0
10	50	3.1 \pm 3.0	20	0

Table 2. Dose ranging using ethanolic solutions of HD

9/23/08 Dose ranging

Histology read initially on 10/01/2008

Animal number	Treatment 25 μL	Comment (from S. McClain)
1	ethanol	Normal with some folliculitis
6	3.2 mg/mL HD	Significant inflammatory infiltrate (subepidermal), necrosis is extensive but not complete
7	3.2 mg/mL HD	Inflammatory infiltrate, epidermal necrosis - edge to edge
9	3.2 mg/mL HD	Inflammatory infiltrate, epidermal necrosis - edge to edge
10	3.2 mg/mL HD	Significant inflammatory infiltrate (subepidermal), necrosis is extensive but not complete
11	1.6 mg/mL HD	Some normal tissue at edge, picnotic nuclei
12	1.6 mg/mL HD	Necrosis is patchy, parakeratosis
13	1.6 mg/mL HD	Inflammatory infiltrate, necrosis
14	1.6 mg/mL HD	Inflammatory infiltrate, edema, most epidermis shows necrosis
15	1.6 mg/mL HD	Inflammatory infiltrate, patchy epidermal necrosis
16	0.8 mg/mL HD	Patchy necrosis, superficial epidermal necrosis
17	0.8 mg/mL HD	Outermost layer shows parakeratosis - patchy superficial epidermal necrosis
18	0.8 mg/mL HD	Minimal injury with some zones of patchy necrosis, parakeratosis
20*	0.8 mg/mL HD	looks normal
21	9.5 mg/mL HD	Epidermal and follicular necrosis, some edema
22	9.5 mg/mL HD	Epidermal and follicular necrosis, some edema, some patches appear less abnormal

(animals 2-5 (ethanol control) and animal 8 (3.2 mg/mL HD) died during anesthesia recovery)

(animal 19 - cloning ring dislodged during treatment)

*animal 20 - no indentation from cloning ring, question HD delivery

Although each group had 5-animals initially, there were a significant number of animals that died during recovery from anesthesia; most of these animals were in the ethanol control group.

Table 3. Cutaneous effect of HD (Oct. 08)

(Groups 1 and 2 used exclusively for histology; other groups were treated with cell suspensions)

October 28, 2008 HD treatment (25 µL 3.2 mg/mL HD in ethanol or 25 µL ethanol)

Animals surviving surgery (indicated are those sites sent for histology 24-hours post treatment)

Group # - exposure	Animal #	sample width (mm)	epidermal necrosis (mm)	pustules (width x depth mm)	dermal depth (mm)	thrombosis	muscle injury	Inflammation (location)	follicular injury (mm)*
1-Ethanol	1		No	No		No	No	No	No
	5		No	No		No	No	No	No
2-HD	6	18.00	11.00	0.65x0.20		none	.75-focal	neural vascular	0.20
	7	16.50	8.75	2.75x0.14	0.25-0.4	No	No	Subcut. fat	0.18
	8	18.00	7.90	0.45x0.25	0.2-0.35	Yes	Yes	fat	0.15
	9	17.00	7.40	3.50x0.18	0.22-0.3	Yes	Yes	fat	0.30*
	10	18.00	15.10	2.40x0.15	0.2-0.25	Yes	No	fat	0.10
4-HD	16	6.75	6.30	2.50x0.14	0.22	Yes	Yes	fat	0.14
	17	7.50	5.00	0.70x0.18	0.28	Yes	NA	fat	NA
	18	6.75	6.10	none		Yes	Yes	fat	NA
	19	5.90	3.75	0.70x0.18	0.35	No	No	fat	NA
	20	6.75	3.75	0.55x0.12	0.25	Yes	Yes	fat	0.13
5-Ethanol	21	4.40	No	No		No	No	No	No
6-HD	26	6.25	4.10	1.25x0.47		Yes	NA	fat	NA
	27	5.25	4.00	1.50x0.19		Yes	NA	fat	0.22*
	28	6.75	6.75	2.15x0.20		Yes	Yes	fat	0.27*
	29	5.50	5.50	None		Yes	Yes	fat	0.27*
	30	6.25	4.50	0.75x0.35		Yes	Yes	fat	NA

* Indicates full length of follicle; NA indicate lack of follicle or muscle to evaluation

Table 4. Formation of human epidermis by SF and UD keratinocytes (Oct. 08 application)

Evaluated at one-month post cell application

Group # - exposure (25 µL)	Animal #	Keratinocytes/fibroblasts	Human Epidermis (Human Involucrin ⁺)
3 – ethanol	2	SF control	Yes
	11	SF control	Yes
	15	SF control	No
4 – 3.2 mg/mL HD	16	SF control	No
	17	SF control	No
	18	SF control	No
	19	SF control	Yes
	20	SF control	No
5 – ethanol	21	UD	Yes
	24	UD	No
	25	UD	Yes
6 – 3.2 mg/mL HD	26	UD	Yes
	27	UD	Yes
	28	UD	Yes
	29	UD	Yes
	30	UD	Yes
Control-ethanol	23	no cells	No

Table 5. Cutaneous effects of HD (Dec. 08)

December 15,2008 exposure

Exposure areas excised and sent for histology December 16, 2008

Exposure (25 µL)	Animal #	Epidermal necrosis (mm)*	Pustule	Thrombosis	Muscle injury	Inflammation	Follicular involvement**
Ethanol	1	0	No	No	No	No	No
	2	0	No	No	No	No	No
	3	0.1/6.5	No	No	No	No	No
	4	0	No	No	No	No	No
	5	0	No	No	No	No	No
	6	0	No	No	No	No	No
	7	0	No	No	No	No	No
	8	0	No	No	No	No	No
	9	0	No	No	No	No	No
	10	0	No	No	No	No	No
	11	0.25/6.4	No	No	No	No	No
	12	0.2/6.75	No	No	No	No	No
	13	0	No	No	No	No	No
	14	0	No	No	No	No	No
	15	0	No	No	No	No	No
3.2 mg/mL HD in Ethanol	16	6.0/6.0	Yes	Yes	1/2 sections	Yes	0.14
	17	7.5/8.6	Yes	Yes	No	Yes	0.20
	18	3.75/6.5	Yes	No	No	No	No
	19	8.0/9.35	Yes	Yes	Yes	Yes	0.20
	21	8.78/8.75	Yes	Yes	No	Yes	0.19
	22	6.5/6.5	No	No	No	No	0.20
	23	7.25/8.75	Yes	Yes	Yes	Yes	0.20
	24	7.85/7.85	Yes	Yes	No	Yes	0.20
	26	8.4/8.4	Yes	No	No	Yes	0.18
	27	8.35/8.35	Yes	Yes	Focal	Yes	0.20
	28	8.3/8.3	Yes	Yes	No	Yes	0.15-0.20
	29	8.25/8.25	Yes	No	No	No	0.23
	30	8.25/10	Yes	Yes	Yes	Yes	0.23

*Indicates necrotic epidermis/biopsy length. ** No injury was the full length of the follicle

Animal 20 was eliminated due to loss of the seal on cloning ring during HD application.

Animal 29 was euthanized due to inability to anesthetize for cell application

Table 6. Formation of human epidermis by SF and UD keratinocytes (Dec. 08 application)

Evaluated at 6-weeks post cell application

Group # - exposure (25 µL)	Animal #	Keratinocyte	Human Epidermis (Human Involucrin ⁺)
1 - Ethanol	2	Neonatal	Yes
	4	Neonatal	Yes
2 - 3.2 mg/mL HD	16	Neonatal	Yes
	17	Neonatal	Yes
3 - Ethanol	6	UD	No
	7	UD	No
	8	UD	No
	9	UD	No
4 - 3.2 mg/mL HD	19	UD	Yes
	21	UD	No
	22	UD	No
	23	UD	No
	24	UD	No
5 - Ethanol	11	SF	Yes
	12	SF	No
	13	SF	No
	14	SF	No
	15	SF	No
6 - 3.2 mg/mL HD	27	SF	No
	26	SF	No
	27	SF	Yes
	28	SF	No
	30	SF	No

Animals 1,3,5, 10, 18 died during recovery on Dec. 16, 2008

Animal 29 died on Dec. 17, 2008

Table 7. Evaluation of plasma (fibrin) gels for transplantation

Cell application 3/19/2008 - euthanized 4/14/2009

Animal #	Cell type	Application type	Animal loss	Human Epidermis (human involucrin+)
1	UD	cell slurry		No
2	UD	cell slurry	Died post-anesthesia	No
3	UD	cell slurry	Died post-anesthesia	No
4	UD	cell slurry	Died post-anesthesia	No
5	UD	cell slurry		No
6	UD	cell slurry		No
7	SF	cell slurry		No
8	SF	cell slurry		No
9	SF	cell slurry		No
10	SF	cell slurry	Died post-anesthesia	No
11	SF	cell slurry	Died post-anesthesia	No
12	UD	plasma gel		No
13	UD	plasma gel		No
14	SF	cell slurry		No
15	SF	cell slurry	Died post-anesthesia	No

APPENDIX 3: GROSS OBSERVATIONS OF WOUNDS

Figure 1. Impact of increasing concentrations of sulfur mustard diluted in ethanol

A – Ethanol (25 μ L)

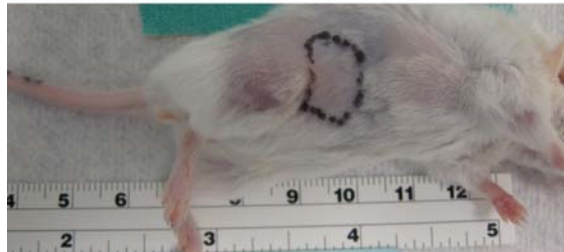
09-24-2008 Dose ranging with XHD in ethanol



Mouse 1: Ethanol control

B - 0.8 mg/mL XHD in ethanol (25 μ L)

9-24-2008 Dose ranging with XHD in ethanol



Mouse 16: 0.8 mg/mL XHD in ethanol



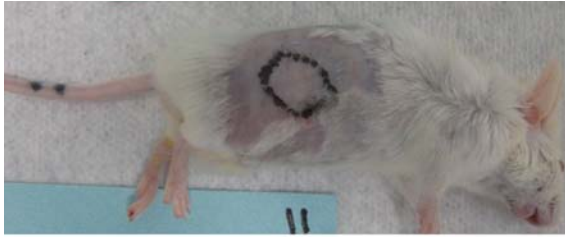
Mouse 17: 0.8 mg/mL XHD in ethanol



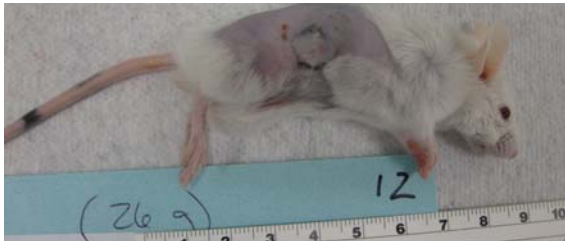
Mouse 18: 0.8 mg/mL XHD in ethanol

C. 1.6 mg/mL XHD in ethanol (25 μ L)

9-24-2008 Dose ranging with XHD in ethanol



Mouse 11: 1.6 mg/mL XHD in ethanol



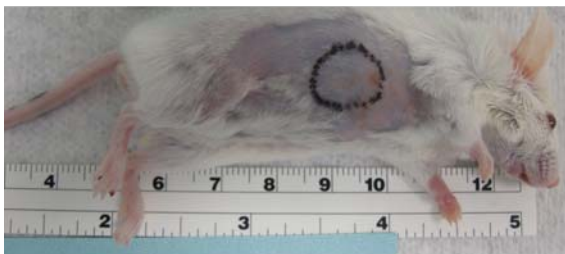
Mouse 12: 1.6 mg/mL XHD in ethanol



Mouse 13: 1.6 mg/mL XHD in ethanol



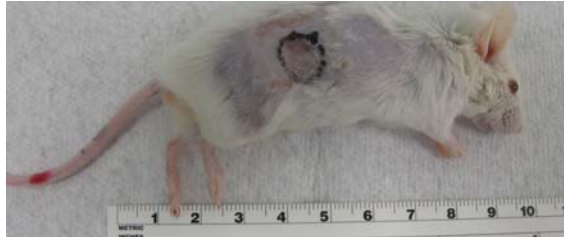
Mouse 14: 1.6 mg/mL XHD in ethanol



Mouse 15: 1.6 mg/mL XHD in ethanol

D. 3.2 mg/mL XHD in ethanol (25 μ L)

9-24-2008 Dose ranging with XHD in ethanol



Mouse 6: 3.2 mg/mL XHD in ethanol



Mouse 7: 3.2 mg/mL XHD in ethanol



Mouse 9: 3.2 mg/mL XHD in ethanol



Mouse 10: 3.2 mg/mL XHD in ethanol

Figure 2. Oct. 08 dosing prior to debridement and keratinocyte spray application

10-28-2008 Dosing



Mouse 1: Ethanol treated



Mouse 7: 3.2 mg/mL XHD in ethanol



Mouse 8: 3.2 mg/mL XHD in ethanol



Mouse 9: 3.2 mg/mL in ethanol



Mouse 10: 3.2 mg/mL XHD in ethanol



Mouse 16: 3.2 mg/mL XHD in ethanol



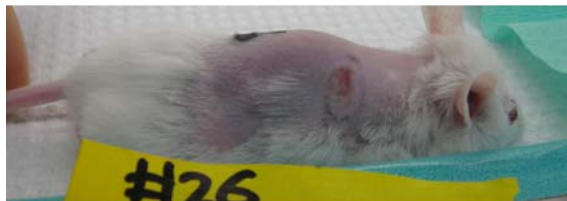
Mouse 17: 3.2 mg/mL XHD in ethanol



Mouse 19: 3.2 mg/mL XHD in ethanol



Mouse 20: 3.2 mg/mL XHD in ethanol



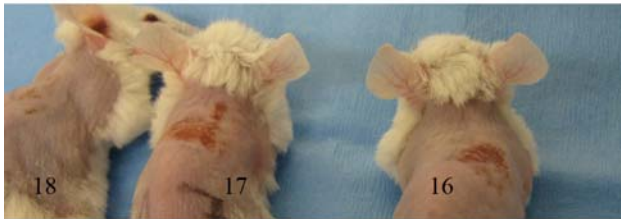
Mouse 26: 3.2 mg/mL XHD in ethanol



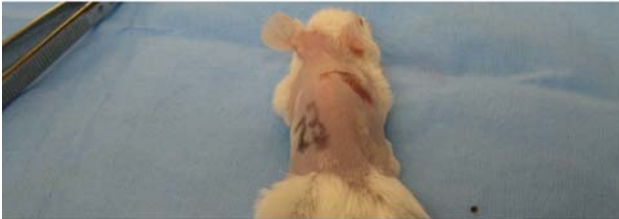
Mouse 27: 3.2 mg/mL XHD in ethanol

Figure 3. . Dec. 08 Dosing with XHD prior to debridement and keratinocyte spray application

12-15-2008 Dosing



Mice 16-18: 3.2 mg/mL XHD in ethanol



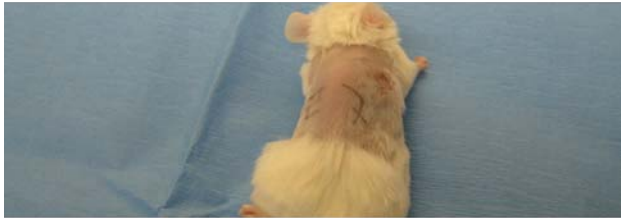
Mouse 23: 3.2 mg/mL XHD in ethanol



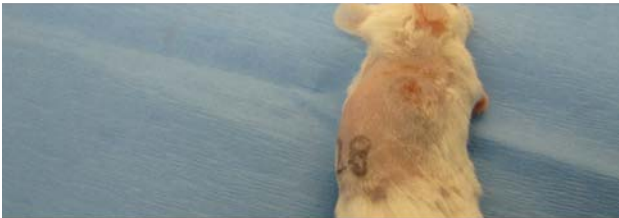
Mouse 24: 3.2 mg/mL XHD in ethanol



Mouse 26: 3.2 mg/mL XHD in ethanol



Mouse 27: 3.2 mg/mL XHD in ethanol



Mouse 28: 3.2 mg/mL XHD in ethanol



Mouse 29: 3.2 mg/mL XHD in ethanol



Mouse 30: 3.2 mg/mL XHD in ethanol

Figure 4. Wound observations 10-day post application of cells (May 2009)

A. Cells supplied as plasma gel construct

5-19-2009 cell application



Mouse 1 on 5-29-2009

5-19-2009 cell application



Mouse 6 on 5-29-2009



Mouse 2 on 5-29-2009



Mouse 7 on 5-29-2009



Mouse 3 on 5-29-2009



Mouse 8 on 5-29-2009



Mouse 4 on 5-29-2009



Mouse 9 on 5-29-2009



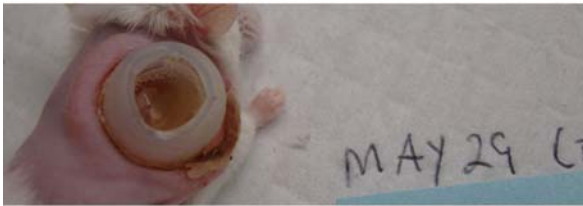
Mouse 5 on 5-29-2009



Mouse 10 on 5-29-2009

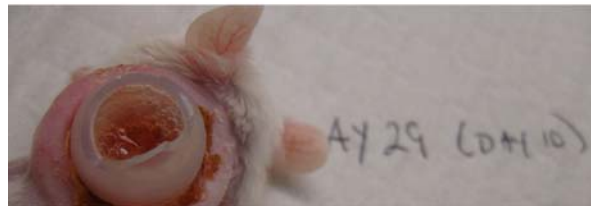
B. Cells supplied as suspensions

5-19-2009 cell application



Mouse 11 on 5-29-2009

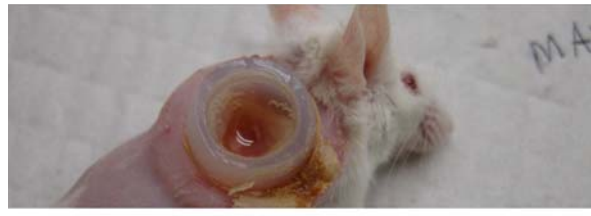
5-19-2009 cell application



Mouse 16 on 5-29-2009



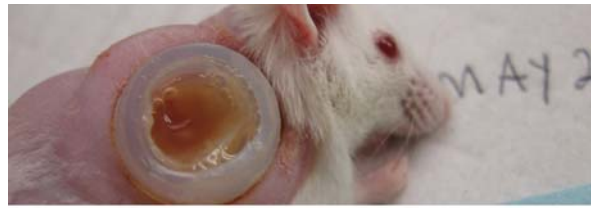
Mouse 12 on 5-29-2009



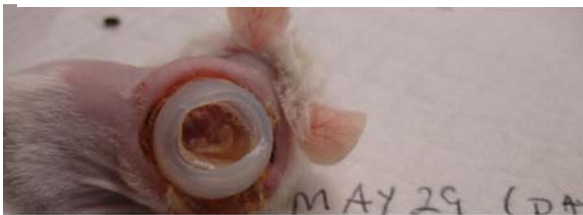
Mouse 17 on 5-29-2009



Mouse 13 on 5-29-2009



Mouse 18 on 5-29-2009



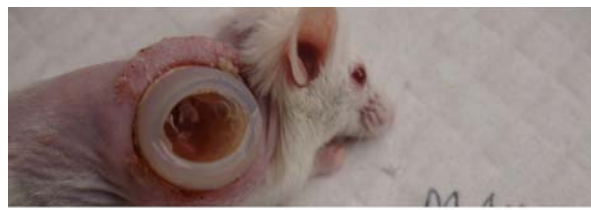
Mouse 14 on 5-29-2009



Mouse 19 on 5-29-2009



Mouse 15 on 5-29-2009



Mouse 20 on 5-29-2009

Referring Physician
Marcia Simon, MD-PhD

Primary Physician
(none specified)

Patient: **SIMONRES, Oct08Treatment**

MRN:

Sex: U Age: N/A DOB: ?

Biopsy Taken: 26-Nov-08

Biopsy Received: 26-Nov-08

Dept Oral Biology & Path
105 Westchester Hall
SUNY, NY 11794-8702
Voice: (n/a)

Site A: HNF

research; 1 mm; Formalin Fixative; 1 block

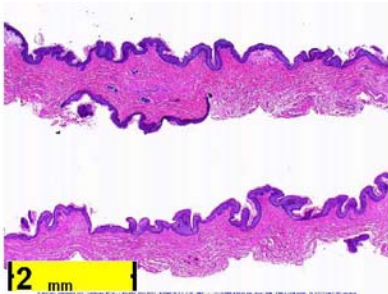
(ICD9: 795.4)

Clinical Impression: HNF (foreskin)

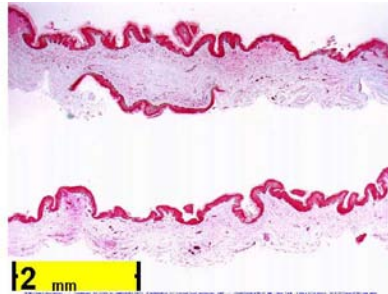
69AF

Microscopic: There is skin near mucous membrane with a sparse infiltrate of lymphocytes. No histopathological changes are found in these sections.

DIAGNOSIS: FORESKIN WITH NO SPECIFIC ABNORMALITY



R16948-08 A 1L1 H&E (1.4X)



R16948-08 A 1L1 Special Stain Involucrin (1.4X)



Site B: 2

research; 1 mm; Formalin Fixative; 1 block

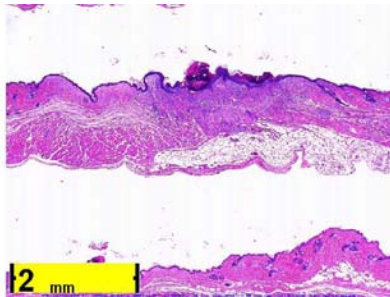
(ICD9: 795.4)

Clinical Impression: 2

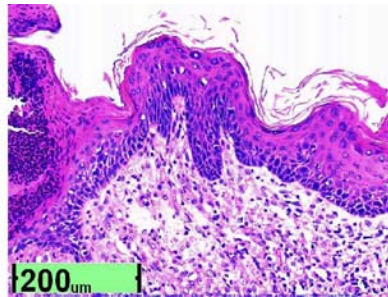
930MH++

Microscopic: Healing wound with hyperkeratosis, presence of granular zone, suprabasal epidermal cells marking with polyclonal human involucrin antibody. Dermal granulomas contain cornified cells, staining with 'human' involucrin antibody.

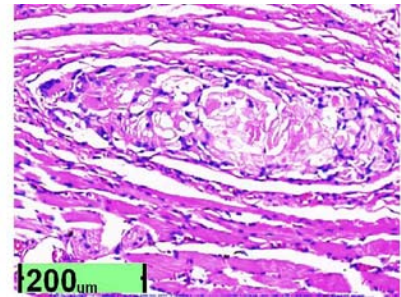
DIAGNOSIS: **HUMAN EPIDERMAL CELLS PRESENT (CONFIRMED WITH 'HUMAN' INVOLUCRIN ANTIBODY)**
SCAR
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)



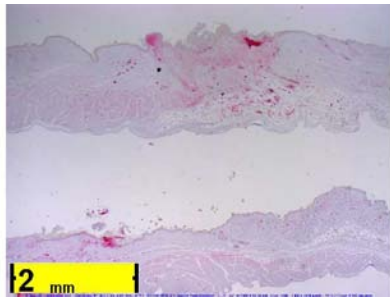
R16948-08 B 1L1 H&E (1.4X)



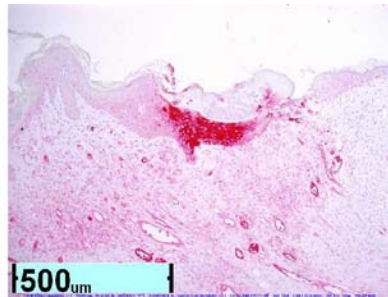
R16948-08 B 1L1 H&E (14X)



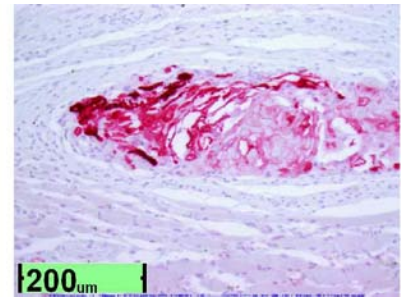
R16948-08 B 1L1 H&E (14X)



R16948-08 B 1L1 Special Stain Involucrin (1.4X)



R16948-08 B 1L1 Special Stain Involucrin (7.2X)



Granuloma around cornified epidermal cells in dermis R16948-08 B 1L1 H&E (14X)



Site C: 11

research; 1 mm; Formalin Fixative; 1 block

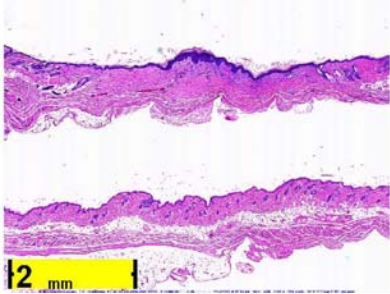
(ICD9: 795.4)

Clinical Impression: 11

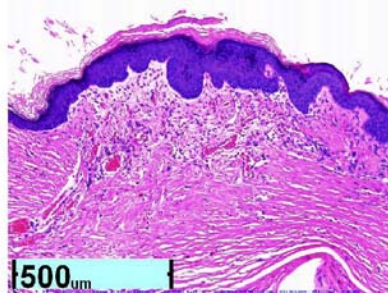
930MH+

Microscopic: Healing wound with hyperkeratosis, repesence of granular zone, suprabasal epidermal cells marking with polyclonal human involucrin antibody. No stainable dermal granulomas are found.

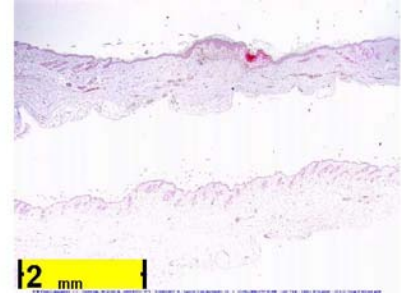
DIAGNOSIS: **HUMAN EPIDERMAL CELLS PRESENT (CONFIRMED BY INVOLUCRIN)**
SCAR
NO DERMAL GRANULOMAS



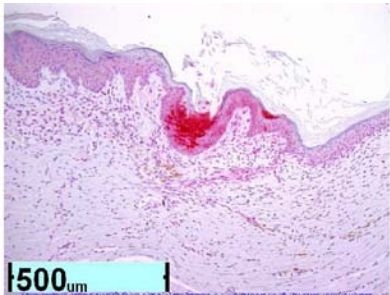
R16948-08 C 1L1 H&E (1.4X)



R16948-08 C 1L1 H&E (7.2X)



R16948-08 C 1L1 Special Stain Involucrin (1.4X)



R16948-08 C 1L1 Special Stain Involucrin (7.2X)



Site D: 15

research; 1 mm; Formalin Fixative; 1 block

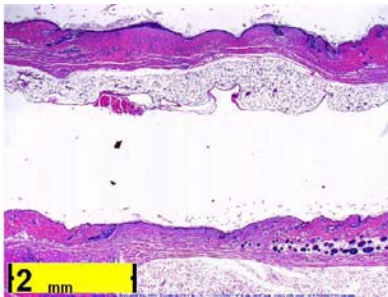
(ICD9: 795.4)

Clinical Impression: 15

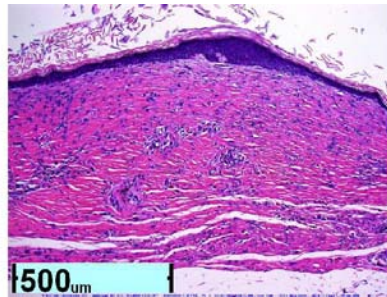
930MH+

Microscopic: Healing wound with hyperplastic epidermis exhibiting NO marking with polyclonal human involucrin antibody. Dermal granulomas, however, contain cornified cells, staining with 'human' involucrin antibody.

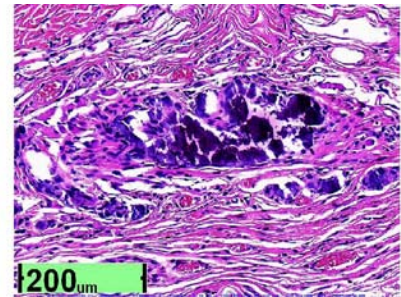
DIAGNOSIS: **EPIDERMIS WITHOUT HUMAN CELLS (CONFIRMED NEGATIVE WITH 'HUMAN' INVOLUCRIN ANTIBODY)**
SCAR
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)



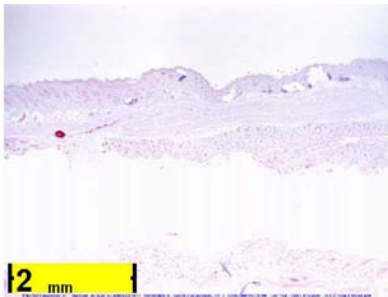
R16948-08 D 1L1 H&E (1.4X)



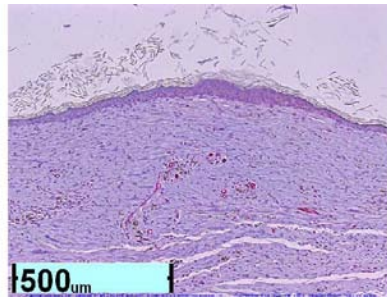
R16948-08 D 1L1 H&E (7.2X)



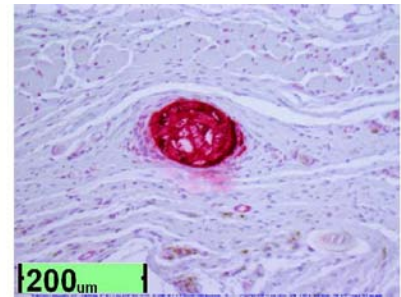
R16948-08 D 1L1 H&E (14X)



R16948-08 D 1L1 Special Stain Involucrin (1.4X)



R16948-08 D 1L1 Special Stain Involucrin (7.2X)



Cluster of cells in deep aspect of healing scar
R16948-08 D 1L1 Special Stain Involucrin (14X)



Site E: 16

research; 1 mm; Formalin Fixative; 1 block

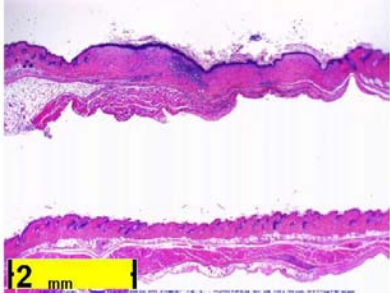
(ICD9: 795.4)

Clinical Impression: 16

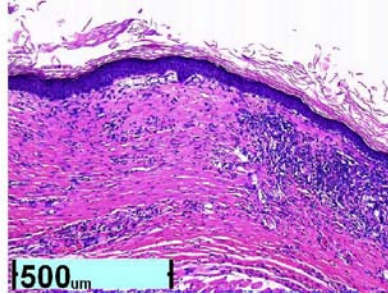
930MH--

Microscopic: Recent or early scar formation with Epidermal hyperplasia; human involucrin **NEGATIVE** in epidermis and dermis.

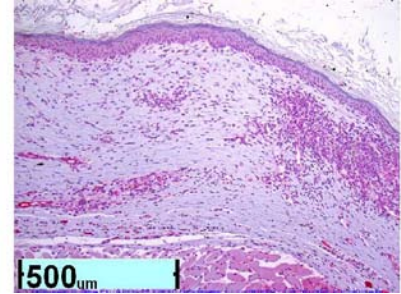
DIAGNOSIS: **SCAR**
NO HUMAN EPIDERMAL CELLS IDENTIFIED
NO GRANULOMAS



R16948-08 E 1L1 H&E (1.4X)



R16948-08 E 1L1 H&E (7.2X)



R16948-08 E 1L1 Special Stain Involucrin (7.2X)



Site F: 17

research; 1 mm; Formalin Fixative; 1 block

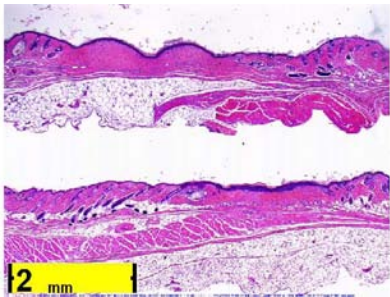
(ICD9: 795.4)

Clinical Impression: 17

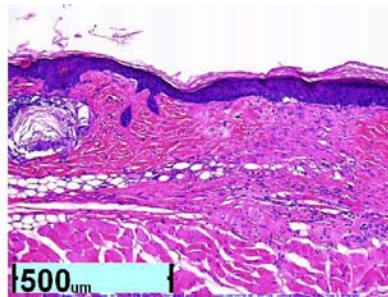
930MH+

Microscopic: Healing wound with hyperplastic epidermis exhibiting NO marking with polyclonal human involucrin antibody. Dermal granulomas, however, contain cornified cells, staining with 'human' involucrin antibody.

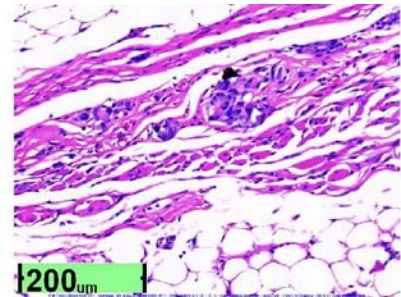
DIAGNOSIS: **EPIDERMIS WITHOUT HUMAN CELLS (CONFIRMED NEGATIVE WITH 'HUMAN' INVOLUCRIN ANTIBODY)**
SCAR
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)



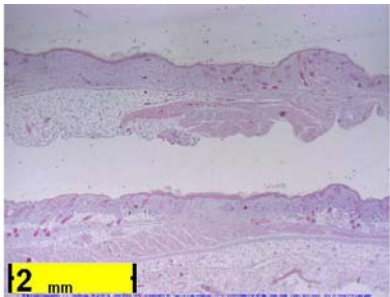
R16948-08 F 1L1 H&E (1.4X)



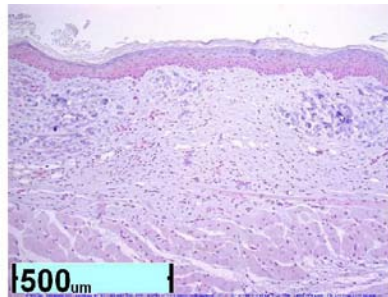
R16948-08 F 1L1 H&E (7.2X)



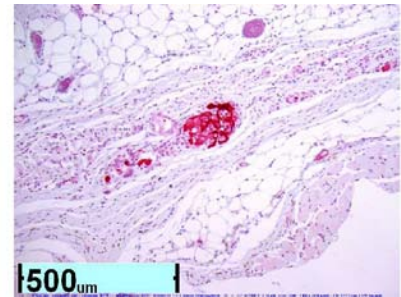
R16948-08 F 1L1 H&E (14X)



R16948-08 F 1L1 Special Stain Involucrin (1.4X)



R16948-08 F 1L1 Special Stain Involucrin (7.2X)



R16948-08 F 1L1 Special Stain Involucrin (7.2X)



Site G: 18

research; 1 mm; Formalin Fixative; 1 block

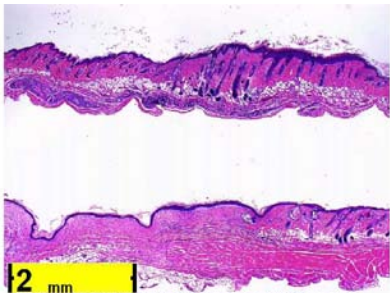
(ICD9: 795.4)

Clinical Impression: 18

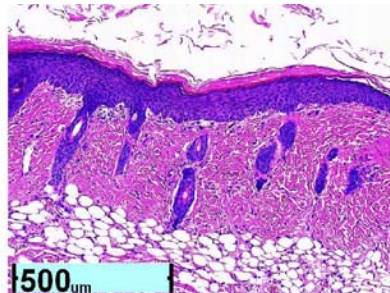
930MH+

Microscopic: Healing wound with hyperplastic epidermis exhibiting NO marking with polyclonal human involucrin antibody. Dermal granulomas, however, contain cornified cells, staining with 'human' involucrin antibody.

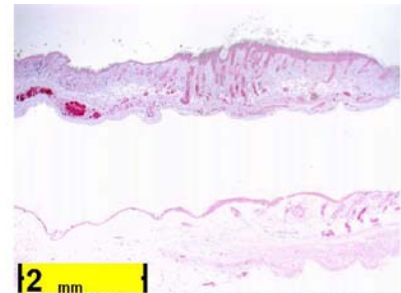
DIAGNOSIS: **EPIDERMIS WITHOUT HUMAN CELLS (CONFIRMED NEGATIVE WITH 'HUMAN' INVOLUCRIN ANTIBODY)**
SCAR
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)



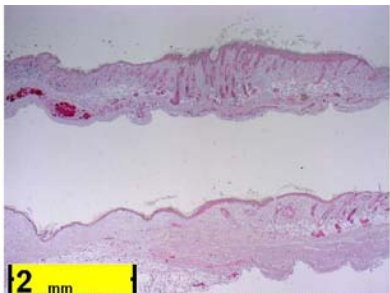
R16948-08 G 1L1 H&E (1.4X)



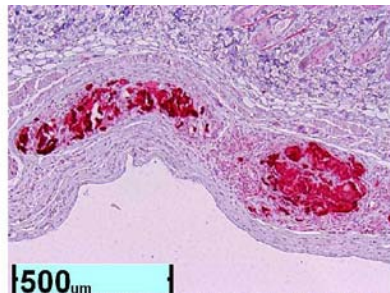
Mouse Epidermal Hyperplasia R16948-08 G 1L1 H&E (7.2X)



R16948-08 G 1L1 Special Stain Involucrin (1.4X)



R16948-08 G 1L1 Special Stain Involucrin (1.4X)



R16948-08 G 1L1 Special Stain Involucrin (7.2X)



Site H: 19

research; 1 mm; Formalin Fixative; 1 block

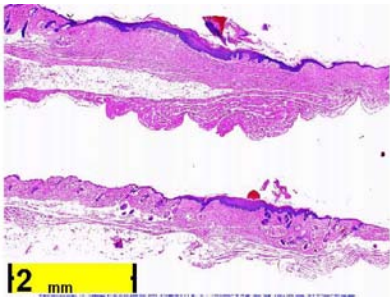
(ICD9: 795.4)

Clinical Impression: 19

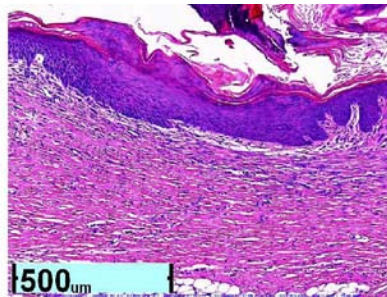
930MH++

Microscopic: Healing wound with hyperkeratosis, presence of granular zone, suprabasal epidermal cells marking with polyclonal human involucrin antibody. Dermal granulomas contain cornified cells, staining with 'human' involucrin antibody.

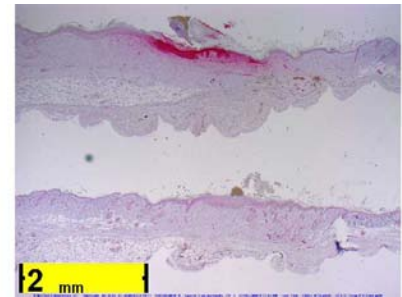
DIAGNOSIS: **HUMAN EPIDERMAL CELLS PRESENT (CONFIRMED WITH 'HUMAN' INVOLUCRIN ANTIBODY)**
SCAR
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)



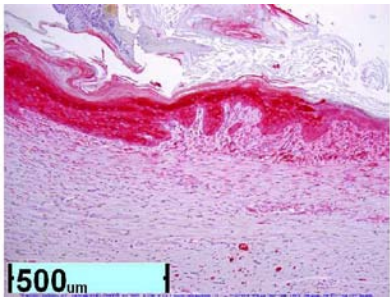
R16948-08 H 1L1 H&E (1.4X)



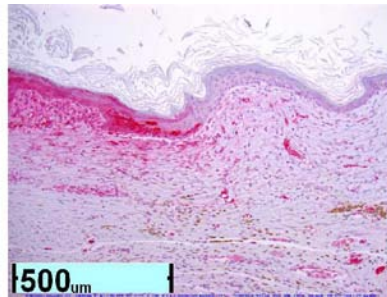
R16948-08 H 1L1 H&E (7.2X)



R16948-08 H 1L1 Special Stain Involucrin (1.4X)



R16948-08 H 1L1 Special Stain Involucrin (7.2X)



R16948-08 H 1L1 Special Stain Involucrin (7.2X)



Site I: 20

research; 1 mm; Formalin Fixative; 1 block

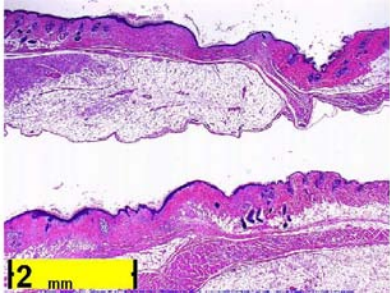
(ICD9: 795.4)

Clinical Impression: 20

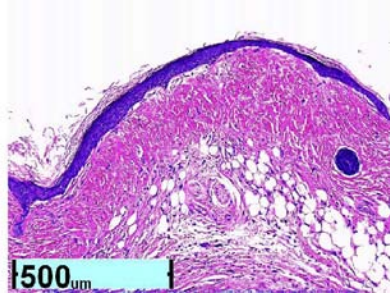
930MH--

Microscopic: Recent or early scar formation with Epidermal hyperplasia; human involucrin **NEGATIVE** in epidermis and dermis.

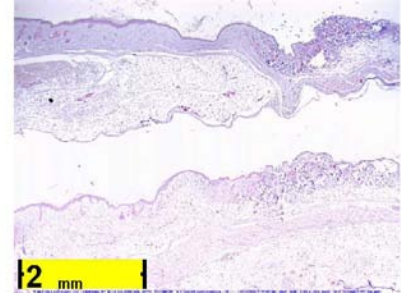
DIAGNOSIS: **SCAR**
NO HUMAN EPIDERMAL CELLS IDENTIFIED
NO GRANULOMAS



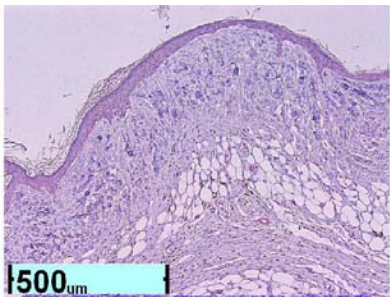
R16948-08 I 1L1 H&E (1.4X)



R16948-08 I 1L1 H&E (7.2X)



R16948-08 I 1L1 Special Stain Involucrin (1.4X)



R16948-08 I 1L1 Special Stain Involucrin (7.2X)



Site J: 21

research; 1 mm; Formalin Fixative; 1 block

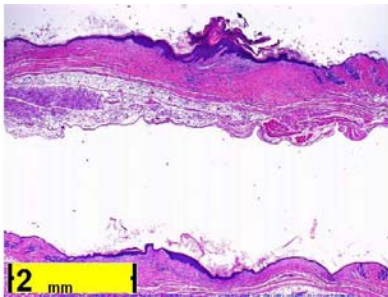
(ICD9: 795.4)

Clinical Impression: 21

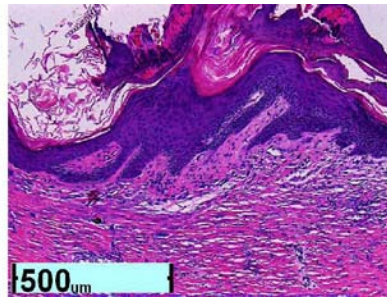
930MH++

Microscopic: Healing wound with hyperkeratosis, presence of granular zone, suprabasal epidermal cells marking with polyclonal human involucrin antibody. Dermal granulomas contain cornified cells, staining with 'human' involucrin antibody.

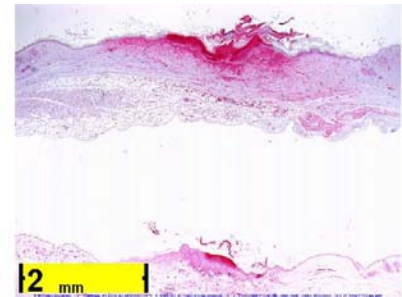
DIAGNOSIS: **HUMAN EPIDERMAL CELLS PRESENT (CONFIRMED WITH 'HUMAN' INVOLUCRIN ANTIBODY)**
SCAR
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)



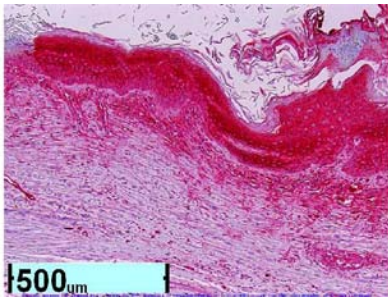
R16948-08 J 1L1 H&E (1.4X)



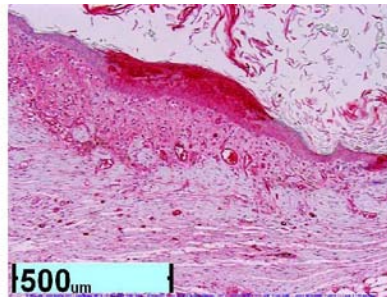
R16948-08 J 1L1 H&E (7.2X)



R16948-08 J 1L1 Special Stain Involucrin (1.4X)



R16948-08 J 1L1 Special Stain Involucrin (7.2X)



R16948-08 J 1L1 Special Stain Involucrin (7.2X)



Site K: 23

research; 1 mm; Formalin Fixative; 1 block

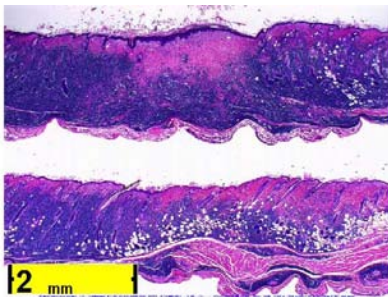
(ICD9: 795.4)

Clinical Impression: 23

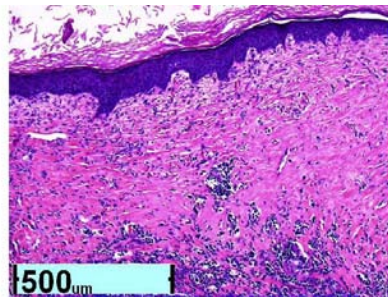
930MHCB-D
ICT

Microscopic: Dense infiltrate of lymphocytes in Mouse skin; Deep Granulomas around cornified cells marking with 'human' involucrin in Healing wound with epidermal hyperplasia; No epidermal staining with 'human' involucrin antibody.

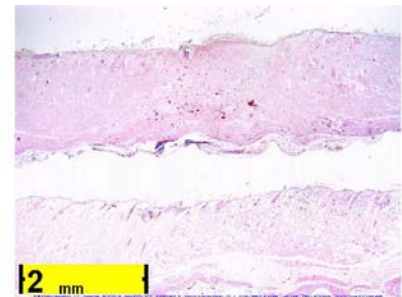
DIAGNOSIS: **NO EPIDERMAL STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY
DENSE INFILTRATE OF LYMPHOCYTES WITH DERMAL SCLEROSIS
CORNIFIED CELL GRANULOMAS STAINING WITH 'HUMAN' INVOLUCRIN
ANTIBODY**



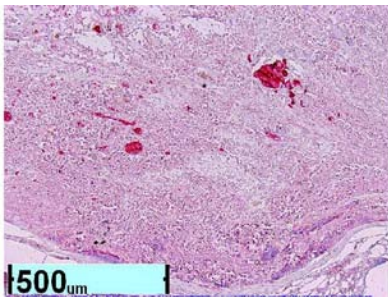
Unusual Dense Infiltrate of Lymphocytes
R16948-08 K 1L1 H&E (1.4X)



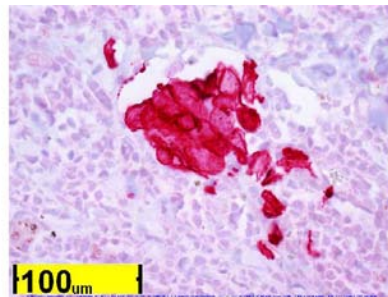
R16948-08 K 1L1 H&E (7.2X)



R16948-08 K 1L1 Special Stain Involucrin (1.4X)



R16948-08 K 1L1 Special Stain Involucrin (7.2X)



R16948-08 K 1L1 Special Stain Involucrin (29X)



Site L: 24

research; 1 mm; Formalin Fixative; 1 block

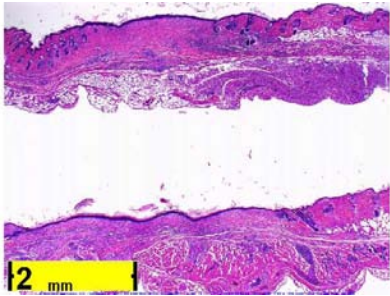
(ICD9: 795.4)

Clinical Impression: 24

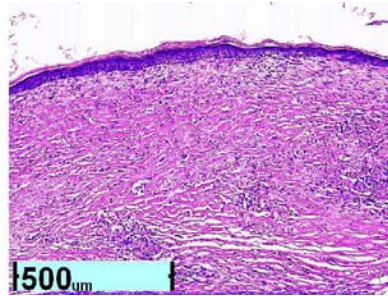
930MH--

Microscopic: Recent or early scar formation with Epidermal hyperplasia; human involucrin **NEGATIVE** in epidermis and dermis.

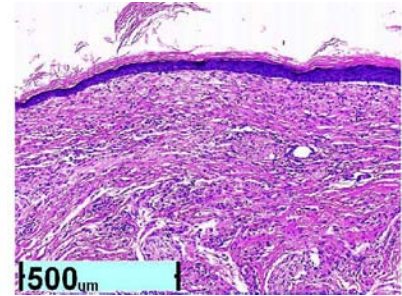
DIAGNOSIS: **SCAR**
NO HUMAN EPIDERMAL CELLS IDENTIFIED
NO GRANULOMAS



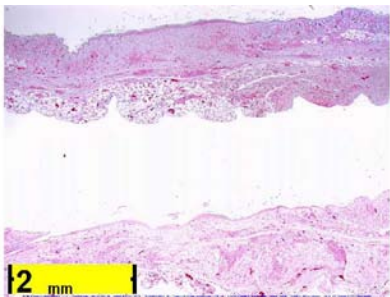
R16948-08 L 1L1 H&E (1.4X)



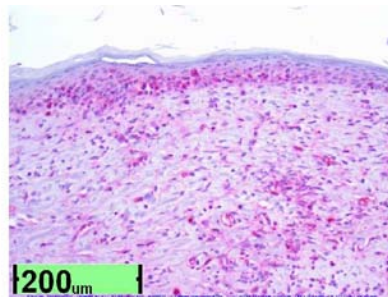
R16948-08 L 1L1 H&E (7.2X)



R16948-08 L 1L1 H&E (7.2X)



R16948-08 L 1L1 Special Stain Involucrin (1.4X)



R16948-08 L 1L1 Special Stain Involucrin (14X)



Site M: 25

research; 1 mm; Formalin Fixative; 1 block

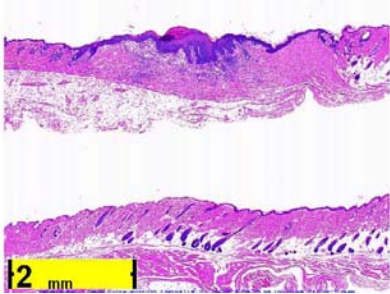
(ICD9: 795.4)

Clinical Impression: 25

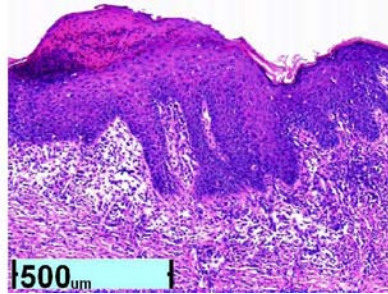
930MH+

Microscopic: Healing wound with hyperkeratosis, repse[n]ce of granular zone, suprabasal epidermal cells marking with polyclonal human involucrin antibody. No stainable dermal granulomas are found.

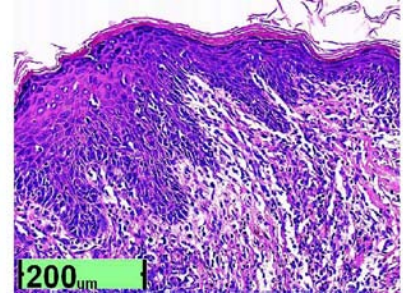
DIAGNOSIS: **HUMAN EPIDERMAL CELLS PRESENT (CONFIRMED BY INVOLUCRIN)
SCAR
NO DERMAL GRANULOMAS**



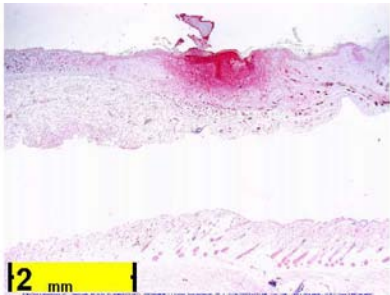
R16948-08 M 1L1 H&E (1.4X)



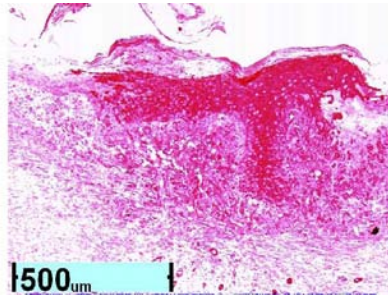
R16948-08 M 1L1 H&E (7.2X)



R16948-08 M 1L1 H&E (14X)



R16948-08 M 1L1 Special Stain Involucrin (1.4X)



R16948-08 M 1L1 Special Stain Involucrin (7.2X)



Site N: 26

research; 1 mm; Formalin Fixative; 1 block

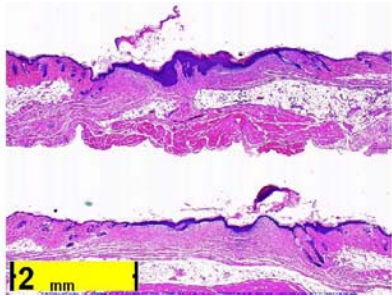
(ICD9: 795.4)

Clinical Impression: 26

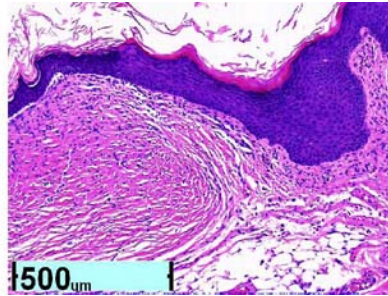
930MH+

Microscopic: Healing wound with hyperkeratosis, repse[n]ce of granular zone, suprabasal epidermal cells marking with polyclonal human involucrin antibody. No stainable dermal granulomas are found.

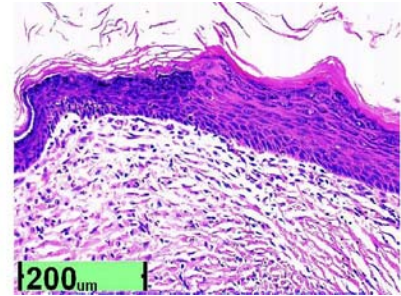
DIAGNOSIS: **HUMAN EPIDERMAL CELLS PRESENT (CONFIRMED BY INVOLUCRIN)
SCAR
NO DERMAL GRANULOMAS**



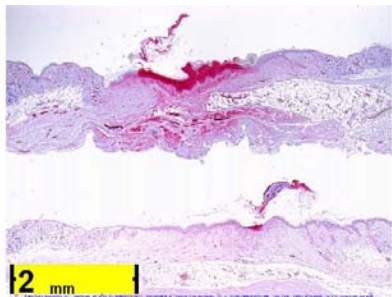
R16948-08 N 1L1 H&E (1.4X)



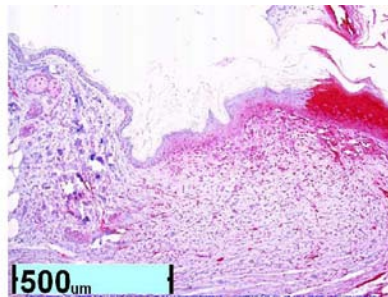
R16948-08 N 1L1 H&E (7.2X)



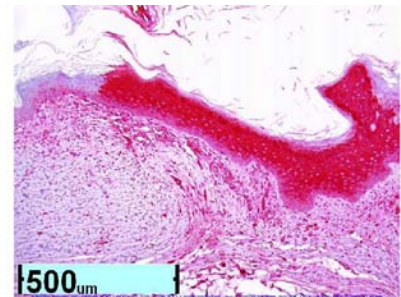
R16948-08 N 1L1 H&E (14X)



R16948-08 N 1L1 Special Stain Involucrin (1.4X)



R16948-08 N 1L1 Special Stain Involucrin (7.2X)



R16948-08 N 1L1 Special Stain Involucrin (7.2X)



Site O: 27

research; 1 mm; Formalin Fixative; 1 block

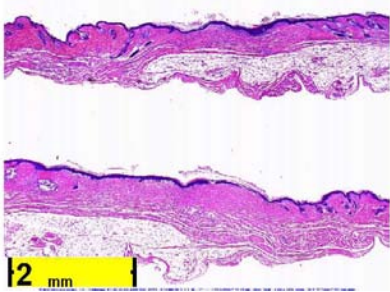
(ICD9: 795.4)

Clinical Impression: 27

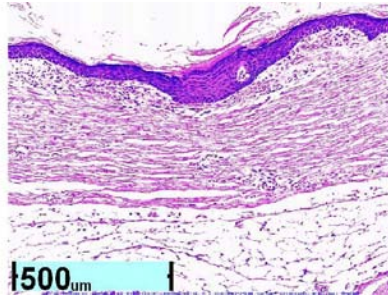
930MH+

Microscopic: Healing wound with hyperkeratosis, repse[n]ce of granular zone, suprabasal epidermal cells marking with polyclonal human involucrin antibody. No stainable dermal granulomas are found.

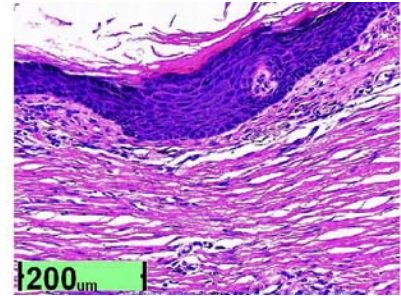
DIAGNOSIS: **HUMAN EPIDERMAL CELLS PRESENT (CONFIRMED BY INVOLUCRIN)**
SCAR
NO DERMAL GRANULOMAS



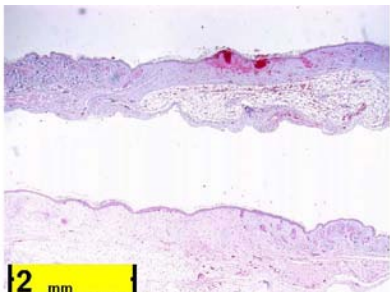
R16948-08 O 1L1 H&E (1.4X)



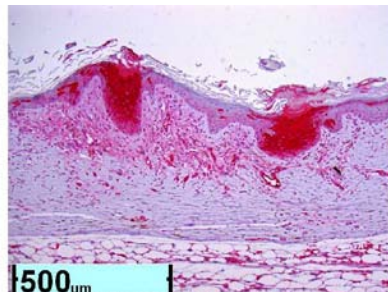
R16948-08 O 1L1 H&E (7.2X)



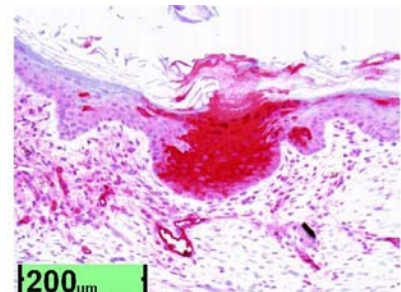
R16948-08 O 1L1 H&E (14X)



R16948-08 O 1L1 Special Stain Involucrin (1.4X)



R16948-08 O 1L1 Special Stain Involucrin (7.2X)



R16948-08 O 1L1 Special Stain Involucrin (14X)



Site P: 28

research; 1 mm; Formalin Fixative; 1 block

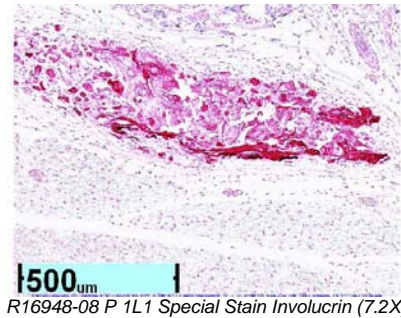
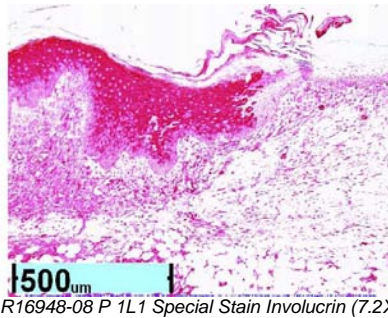
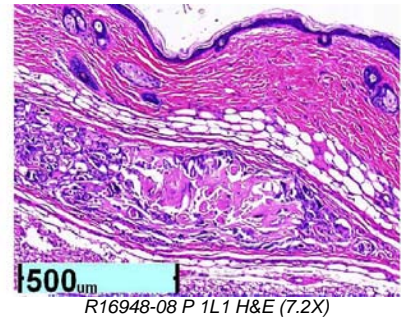
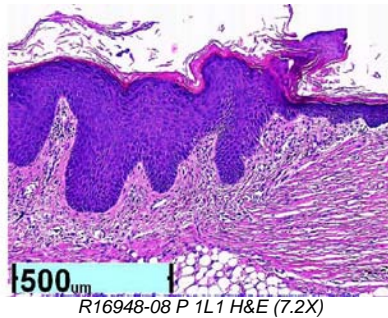
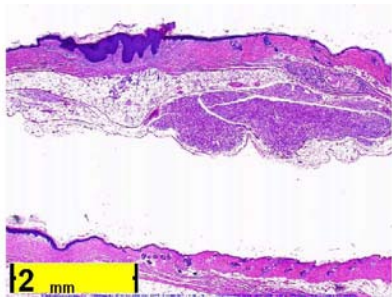
(ICD9: 795.4)

Clinical Impression: 28

930MH++

Microscopic: Healing wound with hyperkeratosis, presence of granular zone, suprabasal epidermal cells marking with polyclonal human involucrin antibody. Dermal granulomas contain cornified cells, staining with 'human' involucrin antibody.

DIAGNOSIS: **HUMAN EPIDERMAL CELLS PRESENT (CONFIRMED WITH 'HUMAN' INVOLUCRIN ANTIBODY)**
SCAR
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)



Site Q: 29

research; 1 mm; Formalin Fixative; 1 block

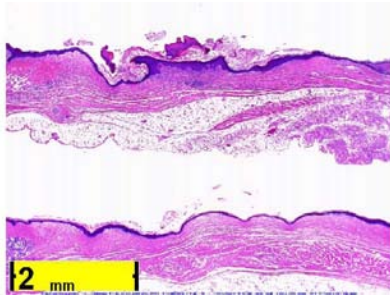
(ICD9: 795.4)

Clinical Impression: 29

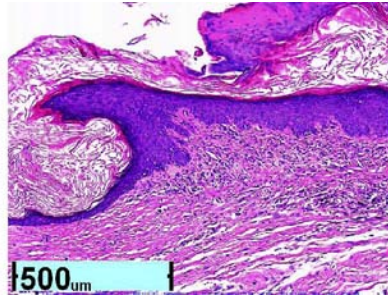
930MH+

Microscopic: Healing wound with hyperkeratosis, repse[n]ce of granular zone, suprabasal epidermal cells marking with polyclonal human involucrin antibody. No stainable dermal granulomas are found.

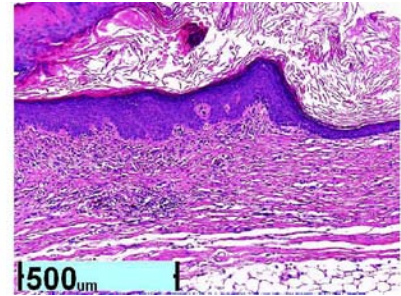
DIAGNOSIS: **HUMAN EPIDERMAL CELLS PRESENT (CONFIRMED BY INVOLUCRIN)
SCAR
NO DERMAL GRANULOMAS**



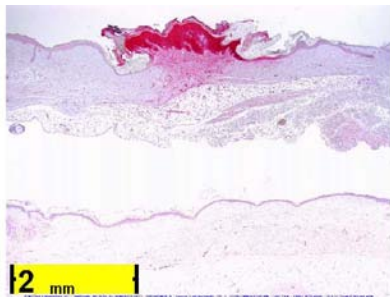
R16948-08 Q 1L1 H&E (1.4X)



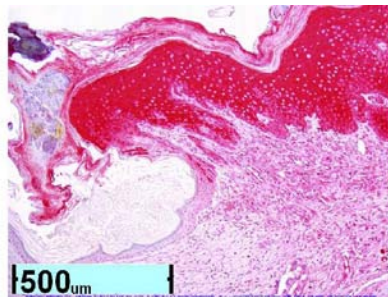
R16948-08 Q 1L1 H&E (7.2X)



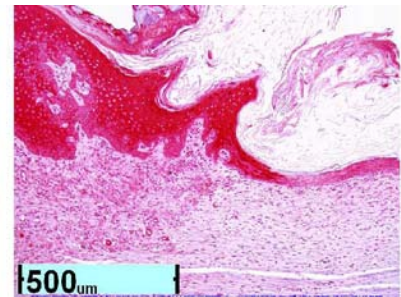
R16948-08 Q 1L1 H&E (7.2X)



R16948-08 Q 1L1 Special Stain Involucrin (1.4X)



R16948-08 Q 1L1 Special Stain Involucrin (7.2X)



R16948-08 Q 1L1 Special Stain Involucrin (7.2X)



Site R: 30

research; 1 mm; Formalin Fixative; 1 block

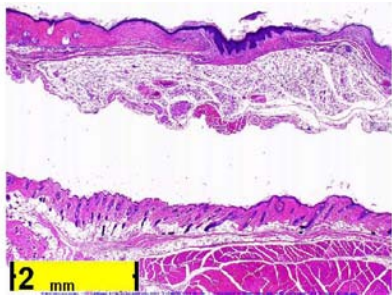
(ICD9: 795.4)

Clinical Impression: 30

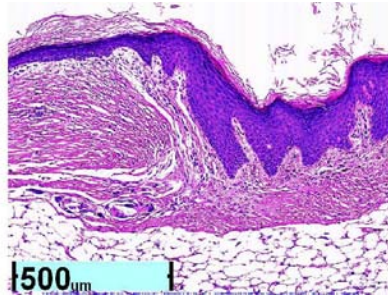
930MH+

Microscopic: Healing wound with hyperkeratosis, repse[n]ce of granular zone, suprabasal epidermal cells marking with polyclonal human involucrin antibody. No stainable dermal granulomas are found.

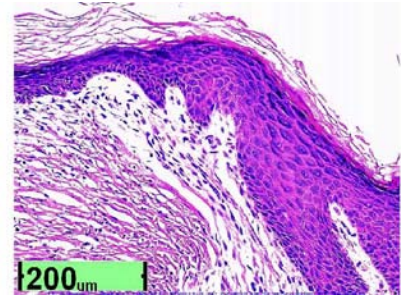
DIAGNOSIS: **HUMAN EPIDERMAL CELLS PRESENT (CONFIRMED BY INVOLUCRIN)
SCAR
NO DERMAL GRANULOMAS**



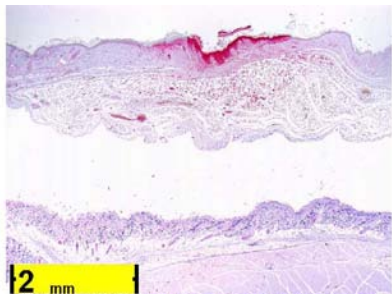
R16948-08 R 1L1 H&E (1.4X)



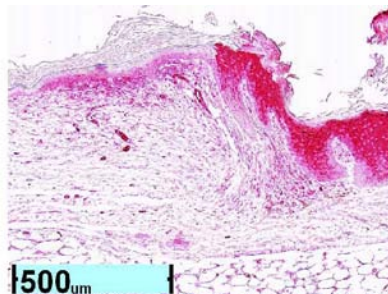
R16948-08 R 1L1 H&E (7.2X)



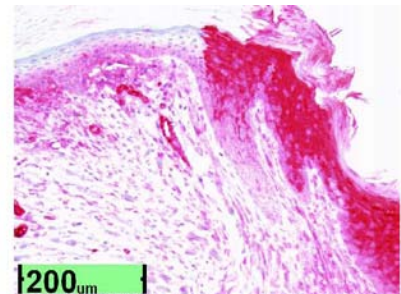
R16948-08 R 1L1 H&E (14X)



R16948-08 R 1L1 Special Stain Involucrin (1.4X)



R16948-08 R 1L1 Special Stain Involucrin (7.2X)



R16948-08 R 1L1 Special Stain Involucrin (14X)

Steve A. McClain MD

Steve A. McClain, M.D.
Electronically signed

this report includes
illustrative color images



Referring Physician
Marcia Simon, MD-PhD
Dept Oral Biology & Path
105 Westchester Hall
SUNY, NY 11794-8702
Voice: (n/a)

Primary Physician
(none specified)

Patient: Kertinocyte Spray, Jan30_2009
MRN:
Sex: U Age: N/A DOB: ?
Biopsy Taken: 30-Jan-09
Biopsy Received: 02-Feb-09

Site A: Mouse 2

research; 1 mm; Form 24H-70% ETOH; 1 block

(ICD9: 795.4)

Clinical Impression: 2

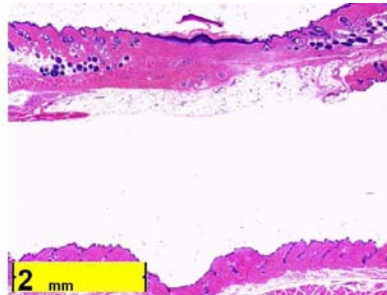
930MH+

Microscopic: Healing wound with hyperkeratosis, represence of granular zone, suprabasal epidermal cells marking with polyclonal human involucrin antibody. No stainable dermal granulomas are found.

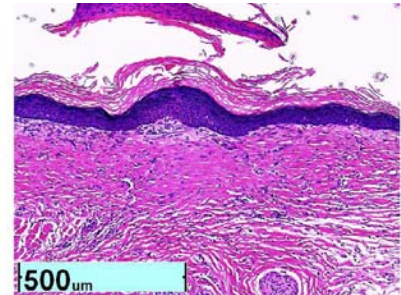
DIAGNOSIS: HUMAN EPIDERMAL CELLS PRESENT (CONFIRMED BY INVOLUCRIN)
SCAR
NO DERMAL GRANULOMAS



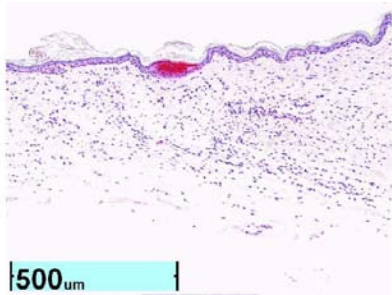
GROSS SPECIMEN IMAGE



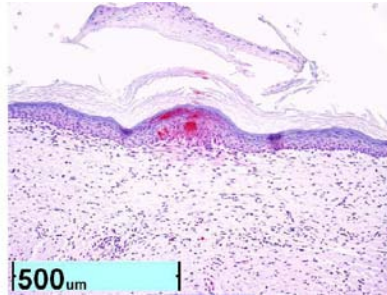
R1299-09 A 1L1 H&E (1.5X)



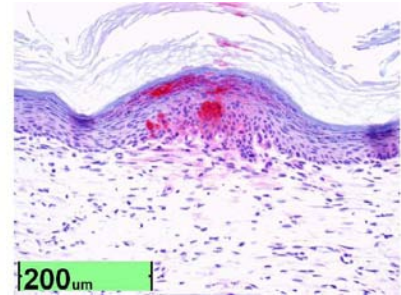
R1299-09 A 1L1 H&E (7.6X)



R1299-09 A 1L1 Special Stain involucrin (7.6X)



R1299-09 A 1L1 H&E (7.6X)



R1299-09 A 1L1 H&E (15X)



Site B: Mouse 4

research; 1 mm; Form 24H-70% ETOH; 1 block

(ICD9: 795.4)

Clinical Impression: 4

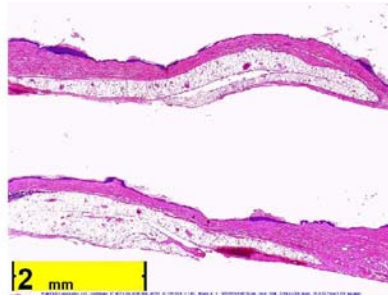
930MH+

Microscopic: Healing wound with hyperkeratosis, repse[n]ce of granular zone, suprabasal epidermal cells marking with polyclonal human involucrin antibody. No stainable dermal granulomas are found.

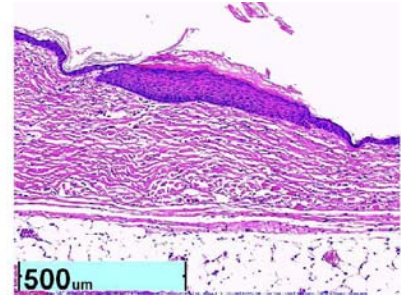
DIAGNOSIS: HUMAN EPIDERMAL CELLS PRESENT (CONFIRMED BY INVOLUCRIN)
SCAR
NO DERMAL GRANULOMAS



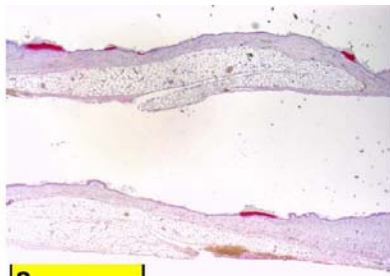
GROSS SPECIMEN IMAGE



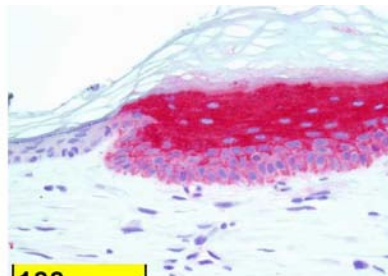
R1299-09 B 1L1 H&E (1.5X)



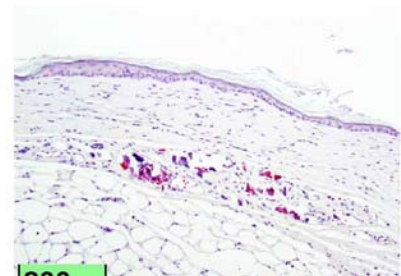
R1299-09 B 1L1 H&E (7.6X)



R1299-09 B 1L1 Special Stain involucrin (1.5X)



R1299-09 B 1L1 Special Stain involucrin (30X)



Focal Granuloma R1299-09 B 1L1 Special Stain involucrin (10X)



Site C: Mouse 6

research; 1 mm; Form 24H-70% ETOH; 1 block

(ICD9: 795.4)

Clinical Impression: 6

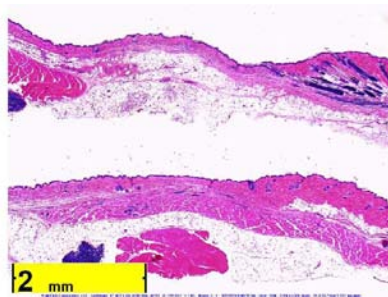
930MH+

Microscopic: Healing wound with hyperplastic epidermis exhibiting NO marking with polyclonal human involucrin antibody. Dermal granulomas, however, contain cornified cells, staining with 'human' involucrin antibody.

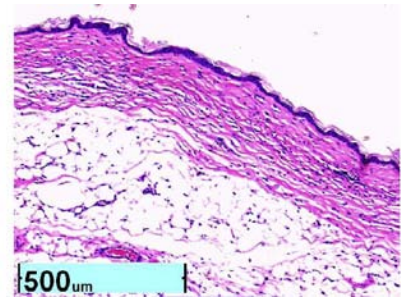
DIAGNOSIS: EPIDERMIS WITHOUT HUMAN CELLS (CONFIRMED NEGATIVE WITH 'HUMAN' INVOLUCRIN ANTIBODY)
SCAR
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)



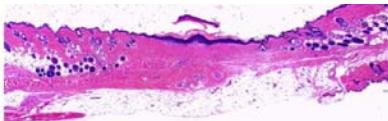
GROSS SPECIMEN IMAGE



R1299-09 C 1L1 H&E (1.5X)



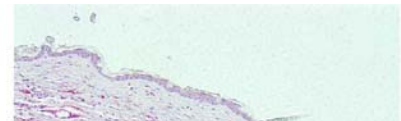
R1299-09 C 1L1 H&E (7.6X)



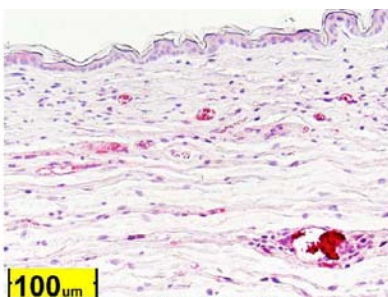
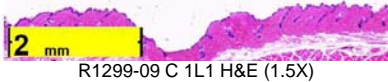
R1299-09 C 1L1 H&E (1.5X)



R1299-09 C 1L1 Special Stain involucrin (1.5X)



R1299-09 C 1L1 Special Stain involucrin (7.5X)



R1299-09 C 1L1 Special Stain involucrin (20X)



Site D: Mouse 7

research; 1 mm; Form 24H-70% ETOH; 1 block

(ICD9: 795.4)

Clinical Impression: 7

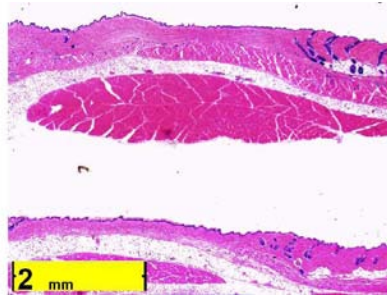
930MH--

Microscopic: Recent or early scar formation with Epidermal hyperplasia; human involucrin NEGATIVE in epidermis and dermis.

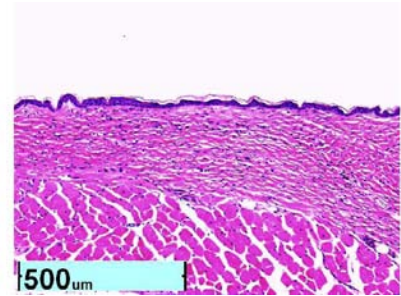
DIAGNOSIS: SCAR
NO HUMAN EPIDERMAL CELLS IDENTIFIED
NO GRANULOMAS



GROSS SPECIMEN IMAGE



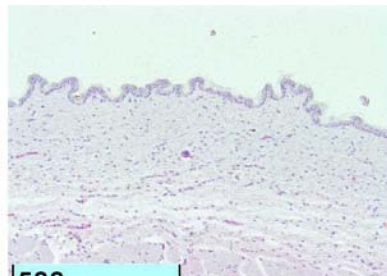
R1299-09 D 1L1 H&E (1.5X)



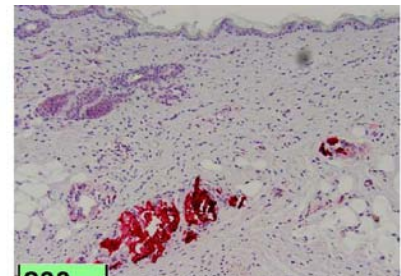
R1299-09 D 1L1 H&E (7.6X)



R1299-09 D 1L1 Special Stain involucrin (1.5X)



R1299-09 D 1L1 Special Stain involucrin (7.6X)



R1299-09 D 1L1 Special Stain involucrin (10X)



Site E: Mouse 8

research; 1 mm; Form 24H-70% ETOH; 1 block

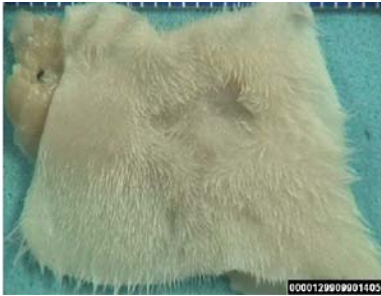
(ICD9: 795.4)

Clinical Impression: 8

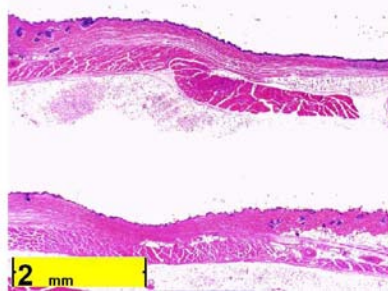
930MH--

Microscopic: Recent or early scar formation with Epidermal hyperplasia; human involucrin **NEGATIVE** in epidermis and dermis.

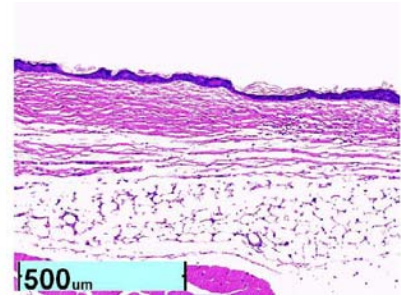
DIAGNOSIS: **SCAR**
NO HUMAN EPIDERMAL CELLS IDENTIFIED
NO GRANULOMAS



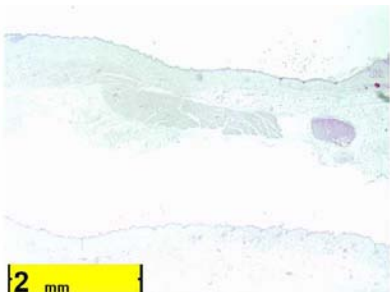
GROSS SPECIMEN IMAGE



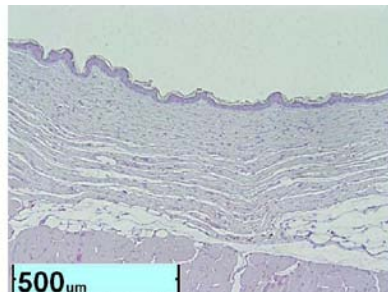
R1299-09 E 1L1 H&E (1.5X)



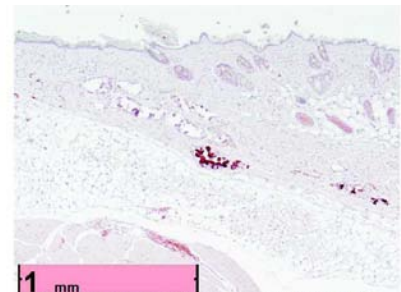
R1299-09 E 1L1 H&E (7.6X)



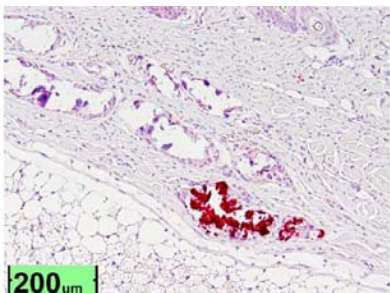
R1299-09 E 1L1 Special Stain involucrin (1.5X)



R1299-09 E 1L1 Special Stain involucrin (7.5X)



R1299-09 E 1L1 Special Stain involucrin (4.1X)



R1299-09 E 1L1 Special Stain involucrin (10X)



Site F: Mouse 9

research; 1 mm; Form 24H-70% ETOH; 1 block

(ICD9: 795.4)

Clinical Impression: 9

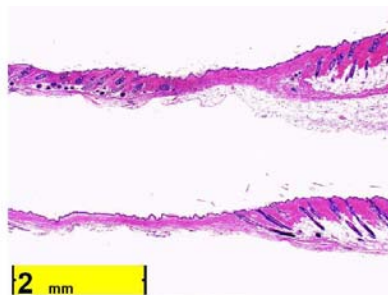
930MH+

Microscopic: Healing wound with hyperplastic epidermis exhibiting NO marking with polyclonal human involucrin antibody. Dermal granulomas, however, contain cornified cells, staining with 'human' involucrin antibody.

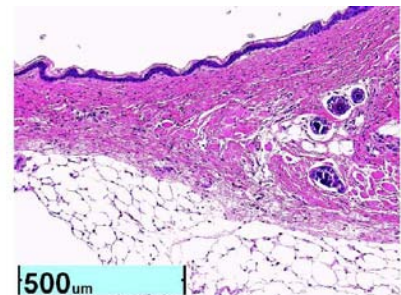
DIAGNOSIS: EPIDERMIS WITHOUT HUMAN CELLS (CONFIRMED NEGATIVE WITH 'HUMAN' INVOLUCRIN ANTIBODY)
SCAR
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)



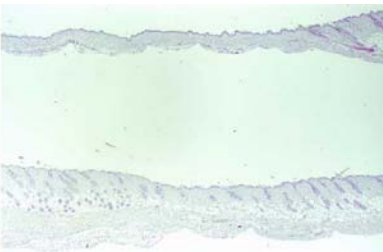
GROSS SPECIMEN IMAGE



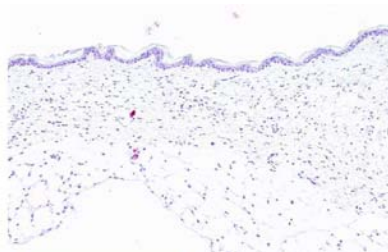
R1299-09 F 1L1 H&E (1.5X)



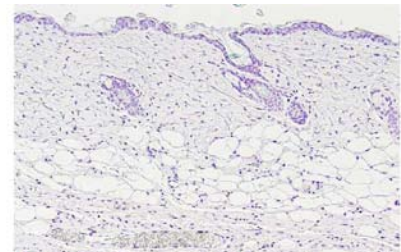
R1299-09 F 1L1 H&E (7.5X)



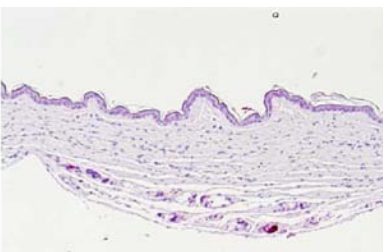
R1299-09 F 1L1 Special Stain involucrin (1.5X)



R1299-09 F 1L1 Special Stain involucrin (7.5X)



R1299-09 F 1L1 Special Stain involucrin (10X)



R1299-09 F 1L1 Special Stain involucrin (10X)



Site G: Mouse 11

research; 1 mm; Form 24H-70% ETOH; 1 block

(ICD9: 795.4)

Clinical Impression: 11

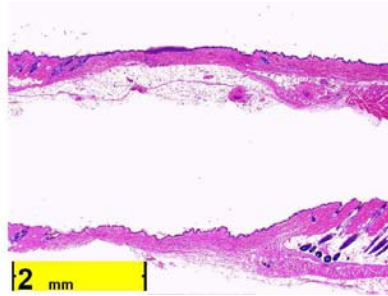
930MH+

Microscopic: Healing wound with hyperkeratosis, reapse of granular zone, suprabasal epidermal cells marking with polyclonal human involucrin antibody. No stainable dermal granulomas are found.

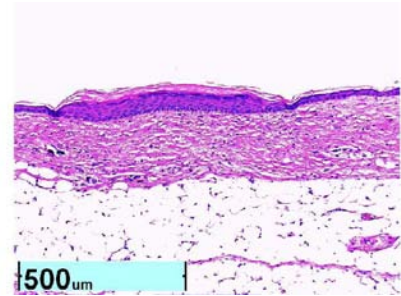
DIAGNOSIS: HUMAN EPIDERMAL CELLS PRESENT (CONFIRMED BY INVOLUCRIN)
SCAR
NO DERMAL GRANULOMAS



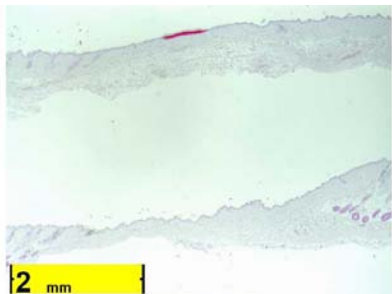
GROSS SPECIMEN IMAGE



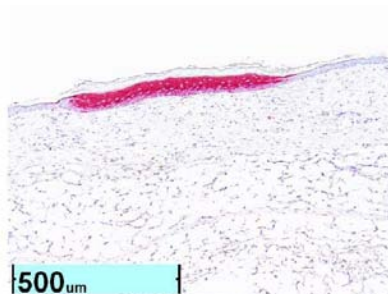
R1299-09 G 1L1 H&E (1.5X)



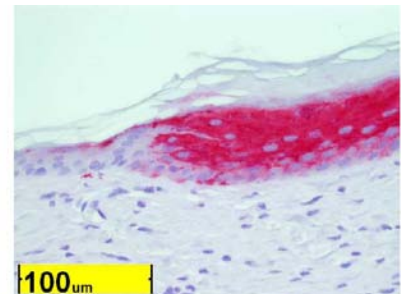
R1299-09 G 1L1 H&E (7.6X)



R1299-09 G 1L1 Special Stain involucrin (1.5X)



R1299-09 G 1L1 Special Stain involucrin (7.6X)



R1299-09 G 1L1 Special Stain involucrin (30X)



Site H: Mouse 12

research; 1 mm; Form 24H-70% ETOH; 1 block

(ICD9: 795.4)

Clinical Impression: 12

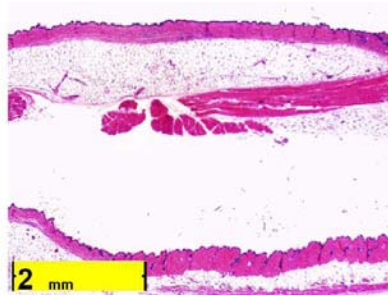
930MH--

Microscopic: Recent or early scar formation with Epidermal hyperplasia; human involucrin **NEGATIVE** in epidermis and dermis.

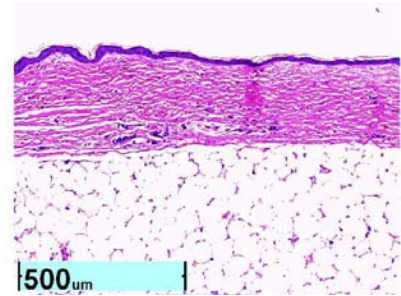
DIAGNOSIS: **SCAR**
NO HUMAN EPIDERMAL CELLS IDENTIFIED
NO GRANULOMAS



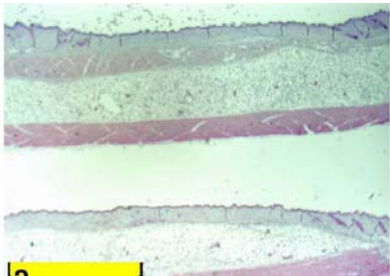
GROSS SPECIMEN IMAGE



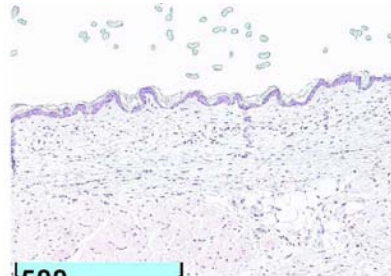
R1299-09 H 1L1 H&E (1.5X)



R1299-09 H 1L1 H&E (7.6X)



R1299-09 H 1L1 Special Stain involucrin (1.5X)



R1299-09 H 1L1 Special Stain involucrin (7.5X)



Site I: Mouse 13

research; 1 mm; Form 24H-70% ETOH; 1 block

(ICD9: 795.4)

Clinical Impression: 13

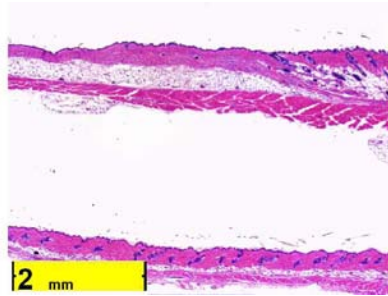
930MH--

Microscopic: Recent or early scar formation with Epidermal hyperplasia; human involucrin **NEGATIVE** in epidermis and dermis.

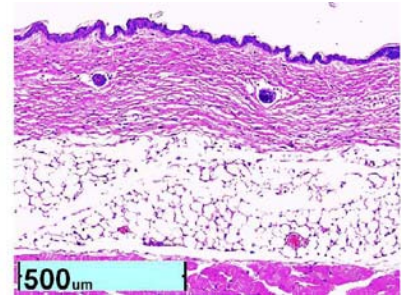
DIAGNOSIS: **SCAR**
NO HUMAN EPIDERMAL CELLS IDENTIFIED
NO GRANULOMAS



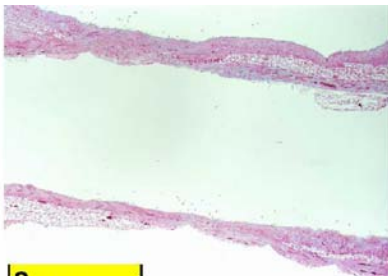
GROSS SPECIMEN IMAGE



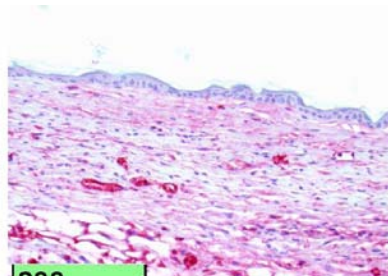
R1299-09 | 1L1 H&E (1.5X)



R1299-09 | 1L1 H&E (7.6X)



R1299-09 | 1L1 Special Stain involucrin (1.5X)



NEGATIVE EPIDERMIS - HIGH BACKGROUND
R1299-09 | 1L1 Special Stain involucrin (15X)



Site J: Mouse 14

research; 1 mm; Form 24H-70% ETOH; 1 block

(ICD9: 795.4)

Clinical Impression: 14

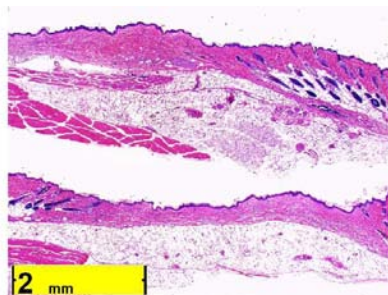
930MH+

Microscopic: Healing wound with hyperplastic epidermis exhibiting NO marking with polyclonal human involucrin antibody. Dermal granulomas, however, contain cornified cells, staining with 'human' involucrin antibody.

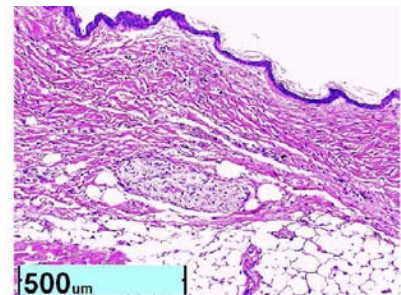
DIAGNOSIS: EPIDERMIS WITHOUT HUMAN CELLS (CONFIRMED NEGATIVE WITH 'HUMAN' INVOLUCRIN ANTIBODY)
SCAR
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)



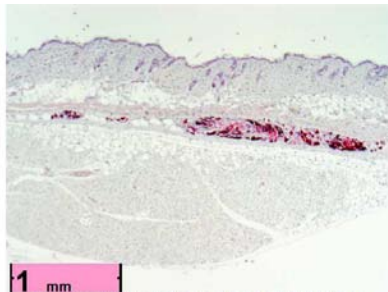
GROSS SPECIMEN IMAGE



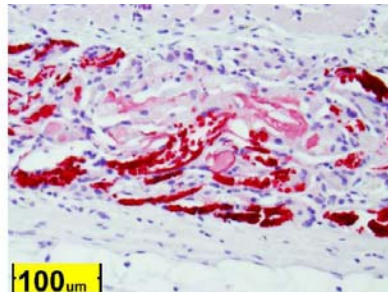
R1299-09 J 1L1 H&E (1.5X)



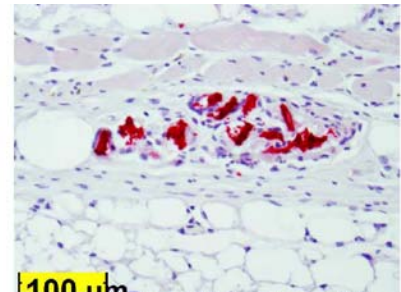
R1299-09 J 1L1 H&E (7.6X)



R1299-09 J 1L1 Special Stain involucrin (2.5X)



R1299-09 J 1L1 Special Stain involucrin (20X)



R1299-09 J 1L1 Special Stain involucrin (20X)



Site K: Mouse 15

research; 1 mm; Form 24H-70% ETOH; 1 block

(ICD9: 795.4)

Clinical Impression: 15

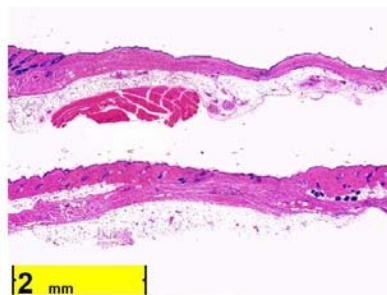
930MH+

Microscopic: Healing wound with hyperplastic epidermis exhibiting NO marking with polyclonal human involucrin antibody. Dermal granulomas, however, contain cornified cells, staining with 'human' involucrin antibody.

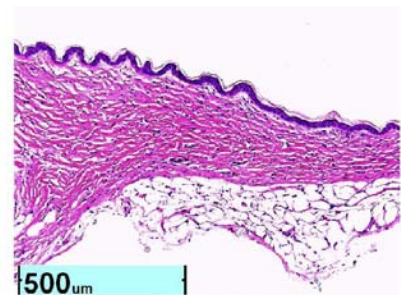
DIAGNOSIS: EPIDERMIS WITHOUT HUMAN CELLS (CONFIRMED NEGATIVE WITH 'HUMAN' INVOLUCRIN ANTIBODY)
SCAR
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)



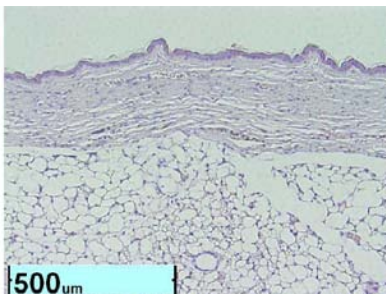
GROSS SPECIMEN IMAGE



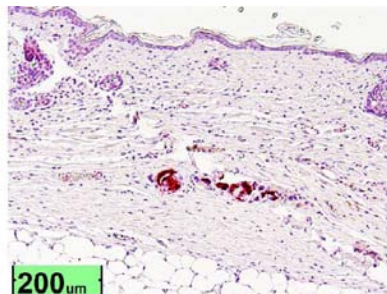
R1299-09 K 1L1 H&E (1.5X)



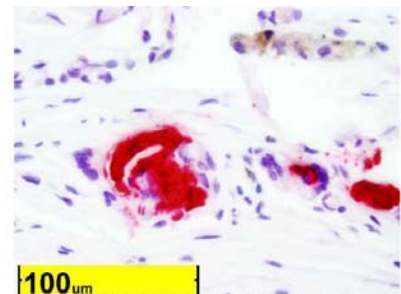
R1299-09 K 1L1 H&E (7.6X)



R1299-09 K 1L1 Special Stain involucrin (7.6X)



R1299-09 K 1L1 Special Stain involucrin (10X)



R1299-09 K 1L1 Special Stain involucrin (41X)



Site L: Mouse 16

research; 1 mm; Form 24H-70% ETOH; 1 block

(ICD9: 795.4)

Clinical Impression: 16

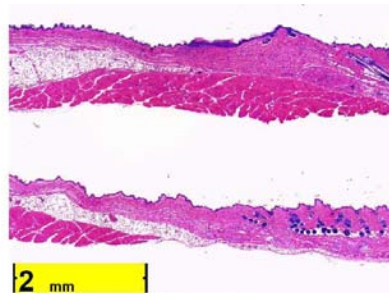
930MH++

Microscopic: Healing wound with hyperkeratosis, presence of granular zone, suprabasal epidermal cells marking with polyclonal human involucrin antibody. Dermal granulomas contain cornified cells, staining with 'human' involucrin antibody.

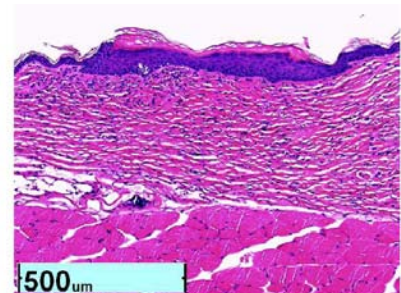
DIAGNOSIS: HUMAN EPIDERMAL CELLS PRESENT (CONFIRMED WITH 'HUMAN' INVOLUCRIN ANTIBODY)
SCAR
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)



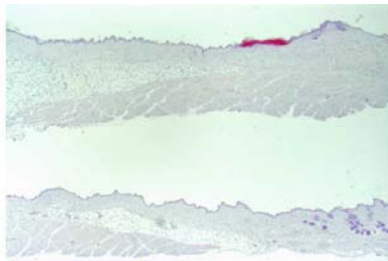
GROSS SPECIMEN IMAGE



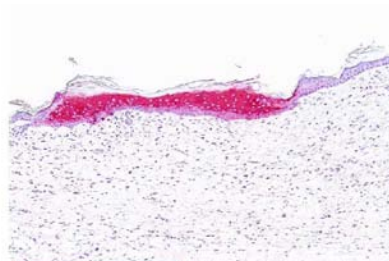
R1299-09 L 1L1 H&E (1.5X)



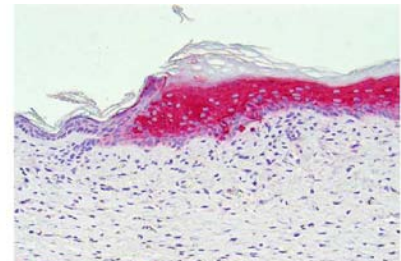
R1299-09 L 1L1 H&E (7.6X)



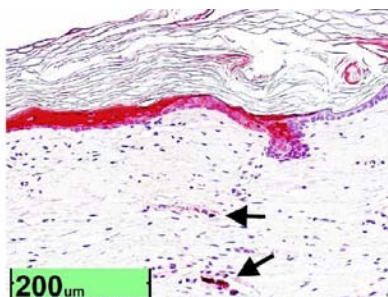
R1299-09 L 1L1 Special Stain Involucrin (1.5X)



R1299-09 L 1L11 Special Stain involucrin (7.6X)



R1299-09 L 1L1 Special Stain Involucrin (15X)



Staining of cornified layer and epidermis; focal granuloma in scar (arrows) R1299-09 L 1L1 Special Stain involucrin (16X)



Site M: Mouse 17

research; 1 mm; Form 24H-70% ETOH; 1 block

(ICD9: 795.4)

Clinical Impression: 17

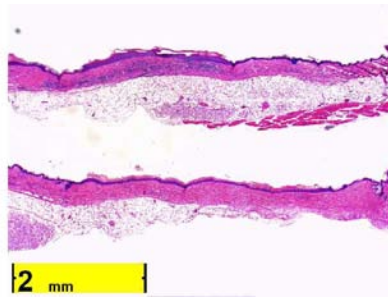
930MH++

Microscopic: Healing wound with hyperkeratosis, presence of granular zone, suprabasal epidermal cells marking with polyclonal human involucrin antibody. Dermal granulomas contain cornified cells, staining with 'human' involucrin antibody.

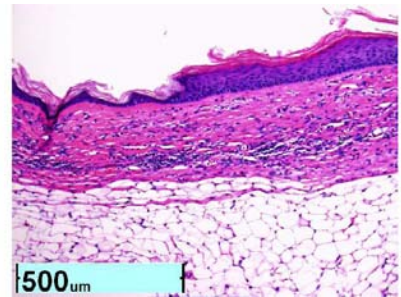
DIAGNOSIS: HUMAN EPIDERMAL CELLS PRESENT (CONFIRMED WITH 'HUMAN' INVOLUCRIN ANTIBODY)
SCAR
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)



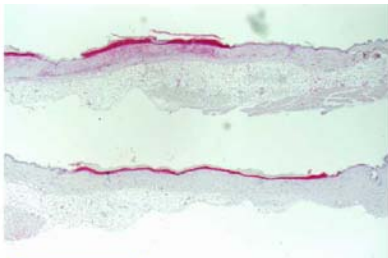
GROSS SPECIMEN IMAGE



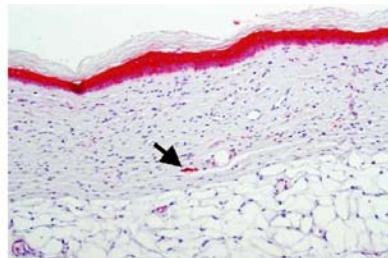
R1299-09 M 1L1 H&E (1.5X)



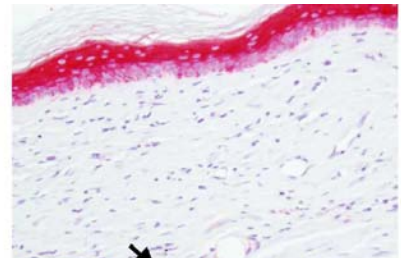
R1299-09 M 1L1 H&E (7.6X)



R1299-09 M 1L1 Special Stain involucrin (1.5X)



R1299-09 M 1L1 Special Stain involucrin (10X)



Focal Granuloma staining in scar (arrowhead)
R1299-09 M 1L1 Special Stain involucrin (21X)



Site N: Mouse 19

research; 1 mm; Form 24H-70% ETOH; 1 block

(ICD9: 795.4)

Clinical Impression: 19

930MH++

Microscopic: Healing wound with hyperkeratosis, presence of granular zone, suprabasal epidermal cells marking with polyclonal human involucrin antibody. Dermal granulomas contain cornified cells, staining with 'human' involucrin antibody.

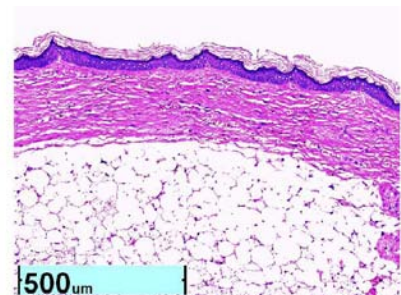
DIAGNOSIS: HUMAN EPIDERMAL CELLS PRESENT (CONFIRMED WITH 'HUMAN' INVOLUCRIN ANTIBODY)
SCAR
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)



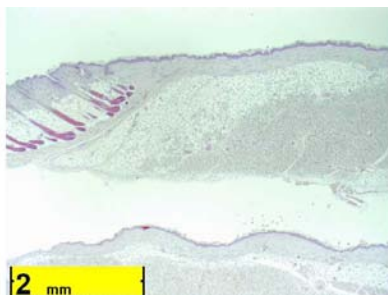
GROSS SPECIMEN IMAGE



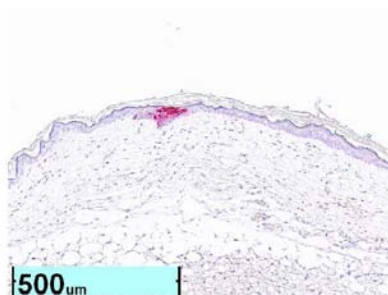
Terminal follicles in SQ fat adjacent to wound (1.5X)



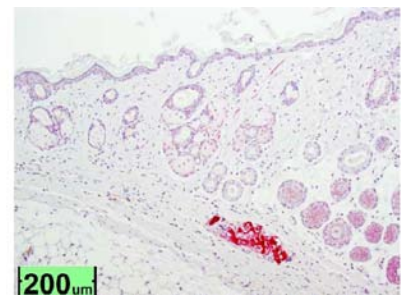
R1299-09 N 1L1 H&E (7.6X)



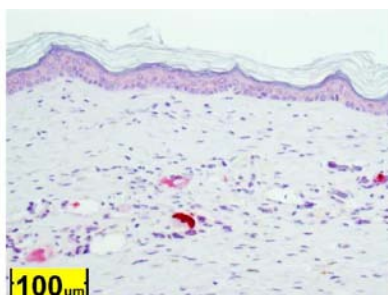
R1299-09 N 1L1 Special Stain involucrin (1.5X)



Focal staining present only on Involucrin
R1299-09 N 1L1 Special Stain involucrin (7.6X)



R1299-09 N 1L1 Special Stain involucrin (8.9X)



R1299-09 N 1L1 Special Stain involucrin (18X)



Site O: Mouse 20

research; 1 mm; Form 24H-70% ETOH; 1 block

(ICD9: 795.4)

Clinical Impression: 20

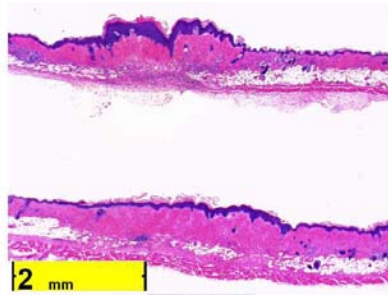
930MH---DI
CT

Microscopic: Recent or early scar formation with hyperkeratosis Epidermal hyperplasia; human involucrin
NEGATIVE in epidermis and dermis.

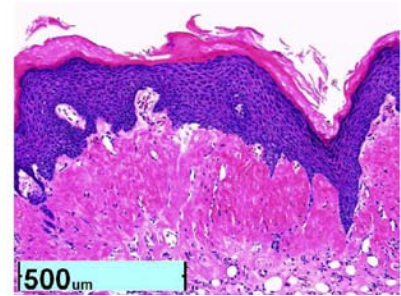
DIAGNOSIS: SCAR WITH SCLEROSIS
NO HUMAN EPIDERMAL CELLS IDENTIFIED
NO GRANULOMAS



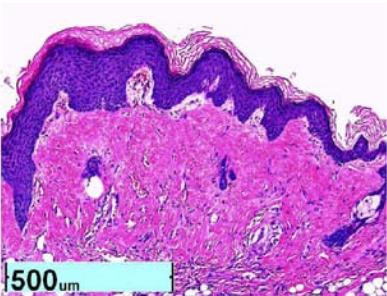
GROSS SPECIMEN IMAGE



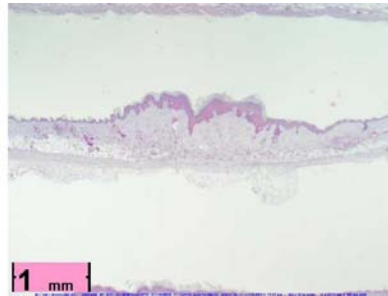
R1299-09 O 1L1 H&E (1.5X)



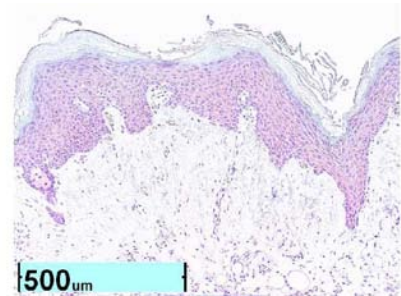
Hyperkeratosis and epidermal hyperplasia above
Dermal sclerosis R1299-09 O 1L1 H&E (7.6X)



Hyperkeratosis and epidermal hyperplasia above
Dermal sclerosis R1299-09 O 1L1 H&E (7.6X)



R1299-09 O 1L1 Special Stain involucrin (1.8X)



R1299-09 O 1L1 Special Stain involucrin (7.6X)



Site P: Mouse 22

research; 1 mm; Form 24H-70% ETOH; 1 block

(ICD9: 795.4)

Clinical Impression: 22

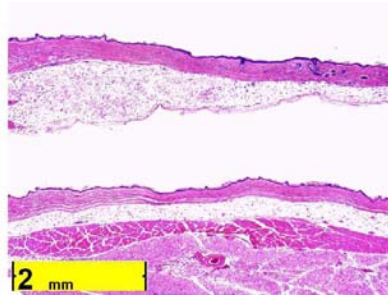
930MH--

Microscopic: Recent or early scar formation with Epidermal hyperplasia; human involucrin NEGATIVE in epidermis and dermis.

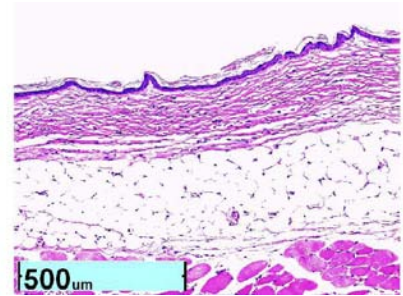
DIAGNOSIS: SCAR
NO HUMAN EPIDERMAL CELLS IDENTIFIED
NO GRANULOMAS



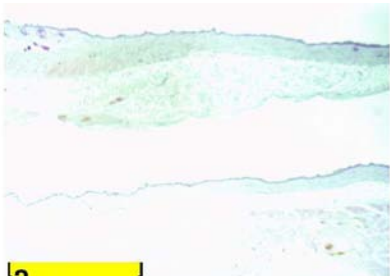
GROSS SPECIMEN IMAGE



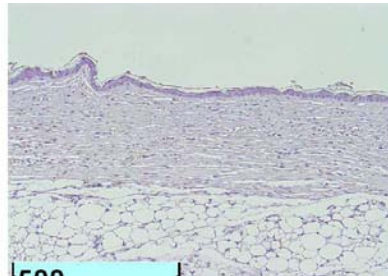
R1299-09 P 1L1 H&E (1.5X)



R1299-09 P 1L1 H&E (7.6X)



R1299-09 P 1L1 Special Stain involucrin (1.5X)



R1299-09 P 1L1 Special Stain involucrin (7.6X)



Site Q: Mouse 23

research; 1 mm; Form 24H-70% ETOH; 1 block

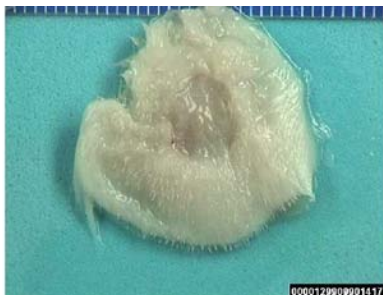
(ICD9: 795.4)

Clinical Impression: 23

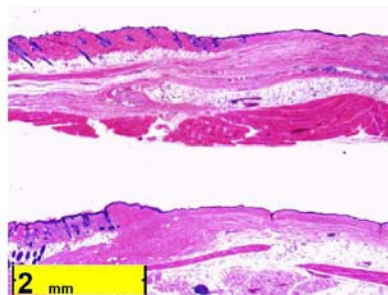
930MH+

Microscopic: Healing wound with hyperplastic epidermis exhibiting NO marking with polyclonal human involucrin antibody. Dermal granulomas, however, contain cornified cells, staining with 'human' involucrin antibody.

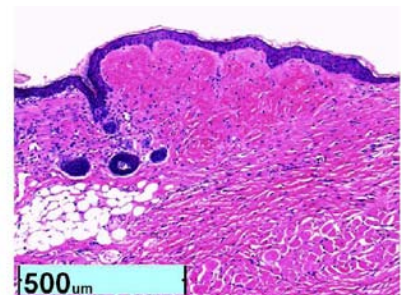
DIAGNOSIS: EPIDERMIS WITHOUT HUMAN CELLS (CONFIRMED NEGATIVE WITH 'HUMAN' INVOLUCRIN ANTIBODY)
SCAR
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)



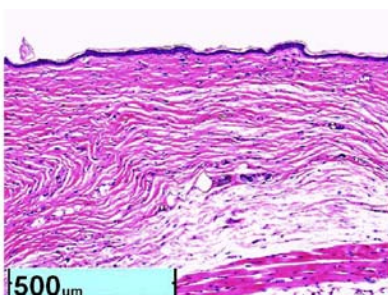
GROSS SPECIMEN IMAGE



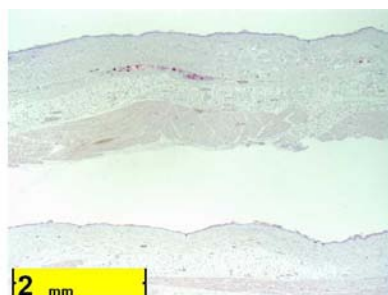
R1299-09 Q 1L1 H&E (1.5X)



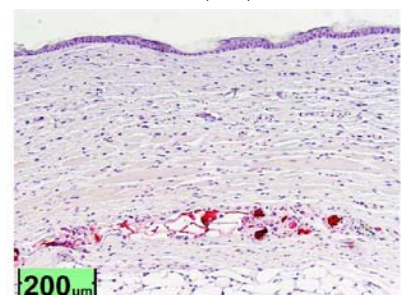
Focal sclerosis at wound edge R1299-09 Q 1L1 H&E (7.6X)



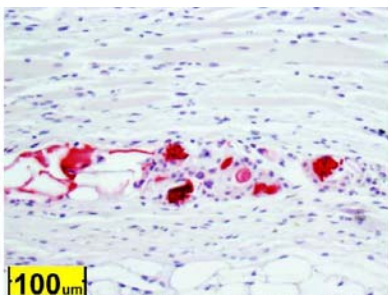
R1299-09 Q 1L1 H&E (7.6X)



R1299-09 Q 1L1 Special Stain involucrin (1.5X)



R1299-09 Q 1L1 Special Stain involucrin (8.9X)



R1299-09 Q 1L1 Special Stain involucrin (18X)



Site R: Mouse 24

research; 1 mm; Form 24H-70% ETOH; 1 block

(ICD9: 795.4)

Clinical Impression: 24

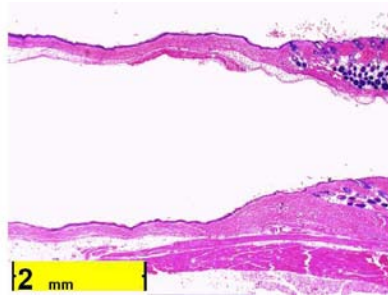
930MH--

Microscopic: Recent or early scar formation with Epidermal hyperplasia; human involucrin **NEGATIVE** in epidermis and dermis.

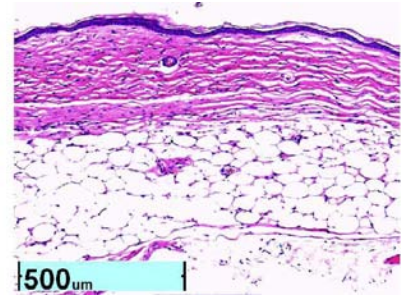
DIAGNOSIS: **SCAR**
NO HUMAN EPIDERMAL CELLS IDENTIFIED
NO GRANULOMAS



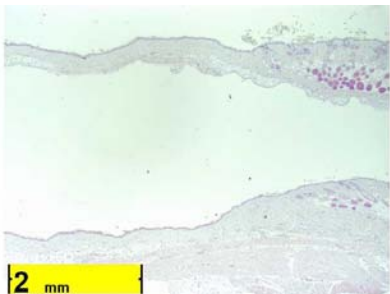
GROSS SPECIMEN IMAGE



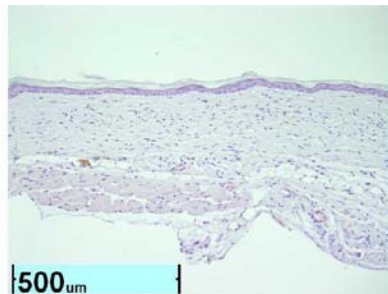
R1299-09 R 1L1 H&E (1.5X)



R1299-09 R 1L1 H&E (7.5X)



R1299-09 R 1L1 Special Stain involucrin (1.5X)



R1299-09 R 1L1 Special Stain involucrin (7.6X)



Site S: Mouse 26

research; 1 mm; Form 24H-70% ETOH; 1 block

(ICD9: 795.4)

Clinical Impression: 26

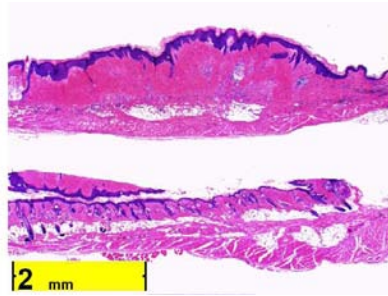
930MH--

Microscopic: Recent or early scar formation with Epidermal hyperplasia; human involucrin NEGATIVE in epidermis and dermis.

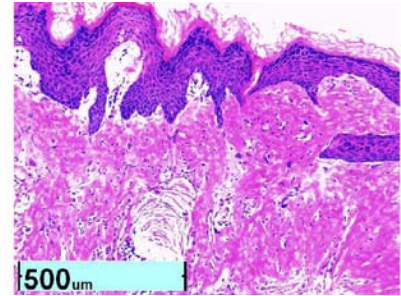
DIAGNOSIS: SCAR
NO HUMAN EPIDERMAL CELLS IDENTIFIED
NO GRANULOMAS



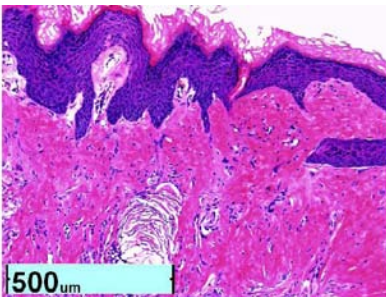
GROSS SPECIMEN IMAGE



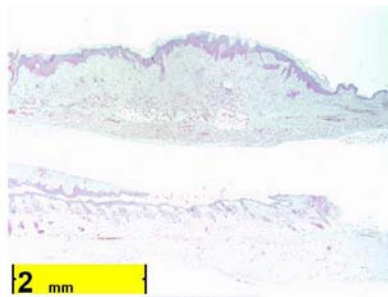
R1299-09 S 1L1 H&E (1.5X)



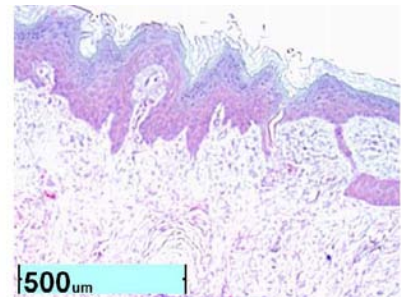
R1299-09 S 1L1 H&E (7.6X)



R1299-09 S 1L1 H&E (7.6X)



R1299-09 S 1L1 Special Stain involucrin (1.5X)



R1299-09 S 1L1 Special Stain involucrin (7.6X)



Site T: Mouse 27

research; 1 mm; Form 24H-70% ETOH; 1 block

(ICD9: 795.4)

Clinical Impression: 27

930MH+

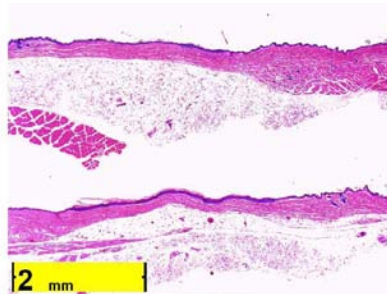
Microscopic: Healing wound with hyperkeratosis, represence of granular zone, suprabasal epidermal cells marking with polyclonal human involucrin antibody. No stainable dermal granulomas are found.

DIAGNOSIS: HUMAN EPIDERMAL CELLS PRESENT (CONFIRMED BY INVOLUCRIN)
SCAR
NO DERMAL GRANULOMAS

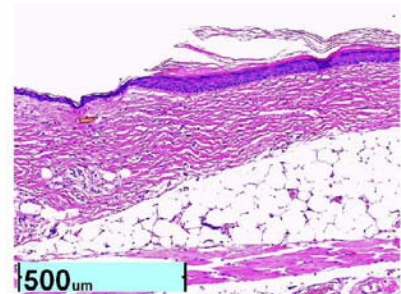
Note: There are focal signs of follicle induction above the healing wound.



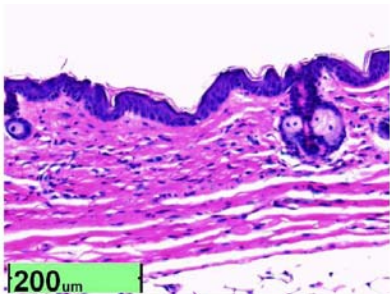
GROSS SPECIMEN IMAGE



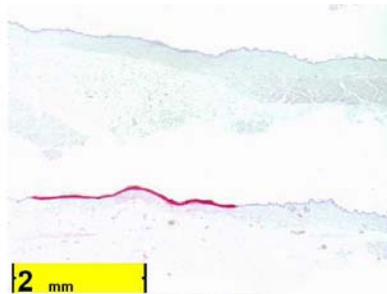
R1299-09 T 1L1 H&E (1.5X)



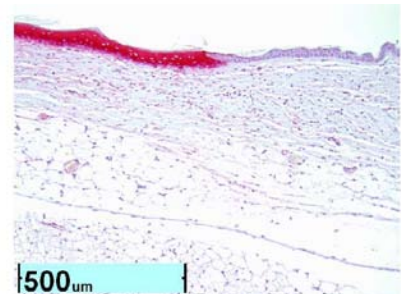
R1299-09 T 1L1 H&E (7.6X)



Follicular induction R1299-09 T 1L1 H&E (15X)



R1299-09 T 1L1 Special Stain involucrin (1.5X)



R1299-09 T 1L1 Special Stain involucrin (7.6X)



Site U: Mouse 28

research; 1 mm; Form 24H-70% ETOH; 1 block

(ICD9: 795.4)

Clinical Impression: 28

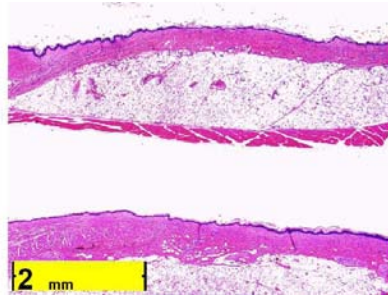
930MH---DI
CT

Microscopic: Recent or early scar formation with Epidermal hyperplasia; human involucrin **NEGATIVE** in epidermis and dermis.

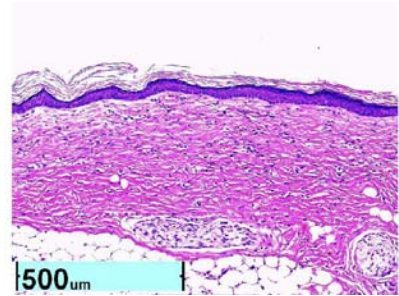
**DIAGNOSIS: SCAR AND DERMAL SCLEROSIS
NO HUMAN EPIDERMAL CELLS IDENTIFIED
NO GRANULOMAS**



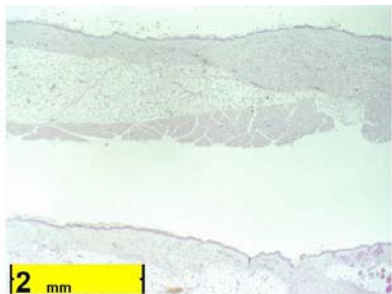
GROSS SPECIMEN IMAGE



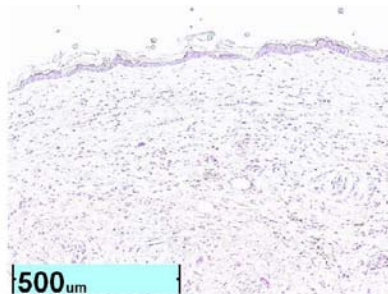
R1299-09 U 1L1 H&E (1.5X)



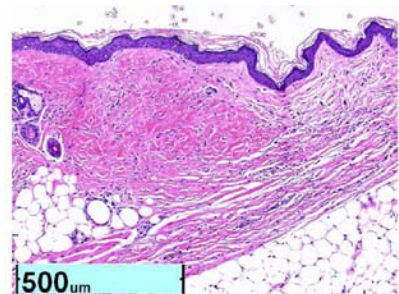
R1299-09 U 1L1 H&E (7.6X)



R1299-09 U 1L1 Special Stain involucrin (1.5X)



R1299-09 U 1L1 Special Stain involucrin (7.6X)



Focal; sclerotic collagen at wound edge R1299-09 U 1L1 H&E (7.6X)



Site V: Mouse 30

research; 1 mm; Form 24H-70% ETOH; 1 block

(ICD9: 795.4)

Clinical Impression: 30

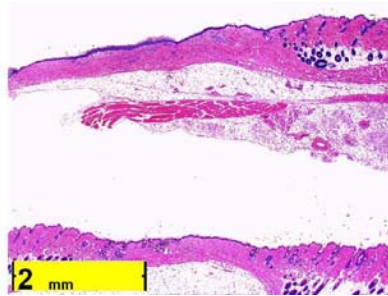
930MH--

Microscopic: Recent or early scar formation with Epidermal hyperplasia; human involucrin **NEGATIVE** in epidermis and dermis.

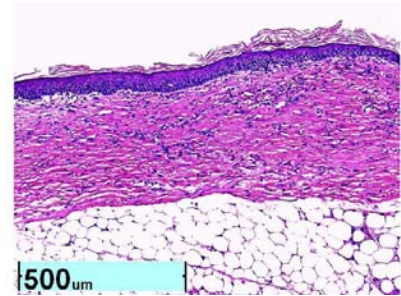
**DIAGNOSIS: SCAR
NO HUMAN EPIDERMAL CELLS IDENTIFIED
NO GRANULOMAS**



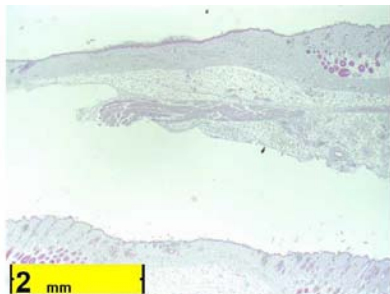
GROSS SPECIMEN IMAGE



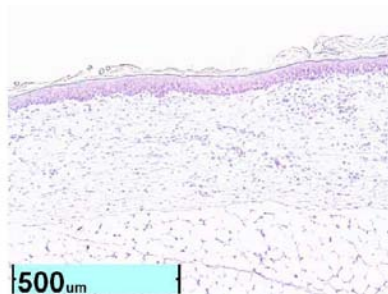
R1299-09 V 1L1 H&E (1.5X)



R1299-09 V 1L1 H&E (7.6X)



R1299-09 V 1L1 Special Stain involucrin (1.5X)



R1299-09 V 1L1 Special Stain involucrin (7.6X)

Steve A. McClain MS

Steve A. McClain, M.D.
Electronically signed

this report includes
illustrative color images



Referring Physician
Marcia Simon, MD-PhD
Dept Oral Biology & Path
105 Westchester Hall
SUNY, NY 11794-8702
Voice: (n/a)

Primary Physician
(none specified)

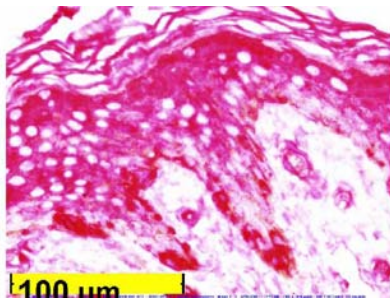
Patient: Simon, Research
MRN:
Sex: U Age: N/A DOB: ?
Biopsy Taken: 20-Apr-09
Biopsy Received: 21-Apr-09

Site A: Mouse 1

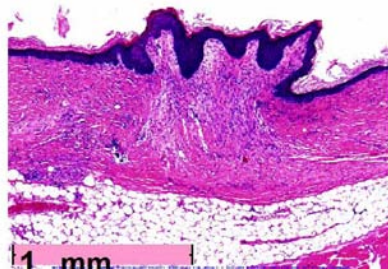
Clinical Impression: research; 1 mm; Form 24H-70% ETOH; 1 block (ICD9: 795.4)
April 20, 2009 930MH--DI
CT

Microscopic: Sclerotic collagen in Healing wound with epidermal hyperplasia and Hyperkeratosis. In the deep aspect of the scarred dermis are granulomas, some containing Human epithelial cells identified by Involucrin stain; other granulomas are calcified.

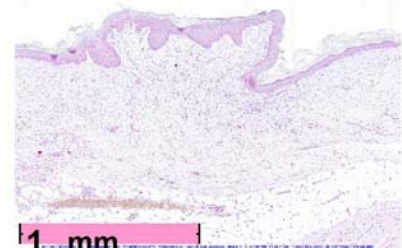
DIAGNOSIS: EPIDERMIS WITHOUT HUMAN CELLS (CONFIRMED NEGATIVE WITH 'HUMAN' INVOLUCRIN ANTIBODY)
DERMAL SCAR AND SCLEROSIS
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)



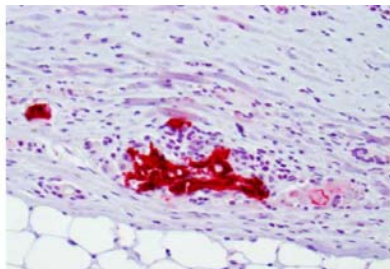
HUMAN FORESKIN CONTROL TISSUE
(Involucrin) R8083-09 A 1L1 Special Stain
Involucrin (39X)



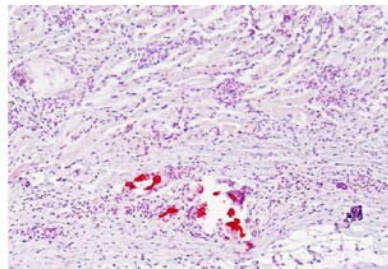
R8083-09 A 1L1 H&E (4.1X)



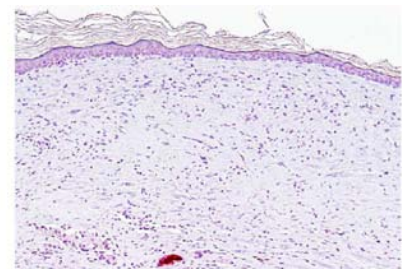
R8083-09 A 1L1 Special Stain Involucrin (4.1X)



R8083-09 A 1L1 Special Stain Involucrin (20X)



R8083-09 A 1L1 Special Stain Involucrin (9.8X)



R8083-09 A 1L1 H&E (9.9X)



Site B: Mouse 5

research; 1 mm; Form 24H-70% ETOH; 1 block

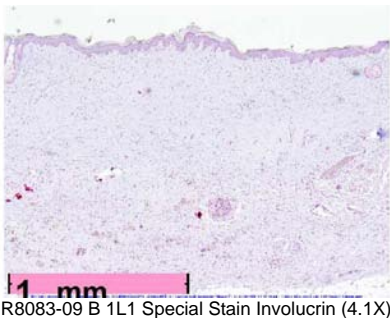
(ICD9: 795.4)

Clinical Impression: April 20, 2009

930SKCG-DI
CT

Microscopic: Sclerotic collagen in Healing wound with epidermal hyperplasia and Hyperkeratosis. In the deep aspect of the scarred dermis is a squamous milium lined by Human epithelial cells identified by Involucrin stain.

DIAGNOSIS: CYST AND DEEP GRANULOMA STAINING WITH "HUMAN" INVOLUCRIN ANTIBODY
COLLAGEN SCLEROSIS
NO EPIDERMAL "HUMAN" INVOLUCRIN STAINING



Site C: Mouse 6

research; 1 mm; Form 24H-70% ETOH; 1 block

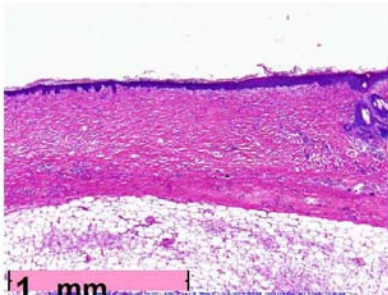
(ICD9: 795.4)

Clinical Impression: April 20, 2009

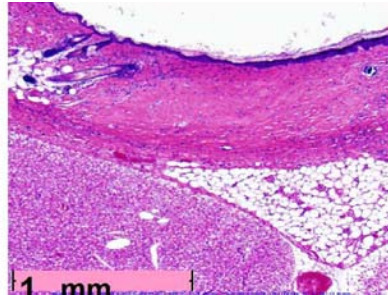
930MH+

Microscopic: Healing wound with hyperplastic epidermis exhibiting NO marking with polyclonal human involucrin antibody. Dermal granulomas, however, contain cornified cells, staining with 'human' involucrin antibody.

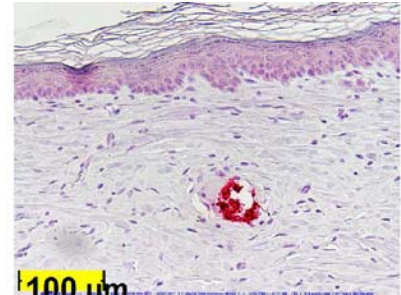
DIAGNOSIS: EPIDERMIS WITHOUT HUMAN CELLS (CONFIRMED NEGATIVE WITH 'HUMAN' INVOLUCRIN ANTIBODY)
SCAR
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)



R8083-09 C 1L1 H&E (4.1X)



R8083-09 C 1L1 H&E (4.1X)



R8083-09 C 1L1 Special Stain Involucrin (20X)



Site D: Mouse 7

research; 1 mm; Form 24H-70% ETOH; 1 block

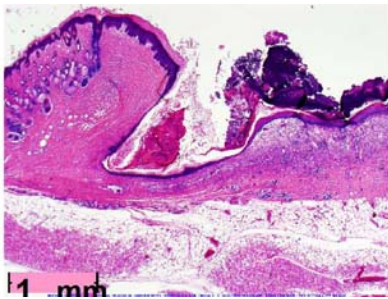
(ICD9: 795.4)

Clinical Impression: April 20, 2009

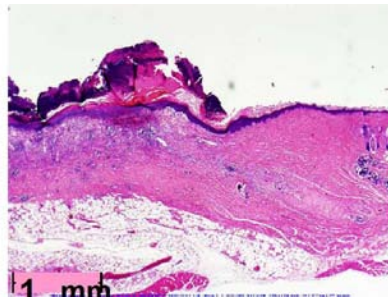
930MH+

Microscopic: Healing wound with hyperplastic epidermis exhibiting NO marking with polyclonal human involucrin antibody. Dermal granulomas, however, contain cornified cells, staining with 'human' involucrin antibody.

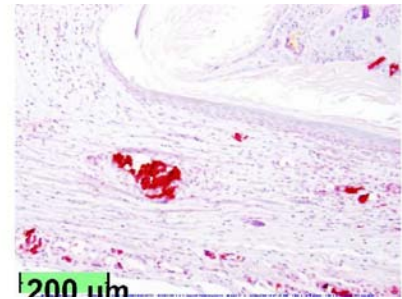
DIAGNOSIS: EPIDERMIS WITHOUT HUMAN CELLS (CONFIRMED NEGATIVE WITH 'HUMAN' INVOLUCRIN ANTIBODY)
SCAR
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)



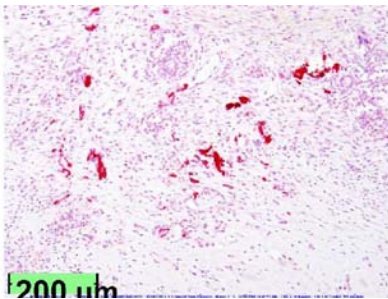
R8083-09 D 1L1 H&E (2X)



R8083-09 D 1L1 H&E (2X)



Cyst wall above granuloma containing Human Involucrin + cells R8083-09 D 1L1 Special Stain Involucrin (10X)



R8083-09 D 1L1 Special Stain Involucrin (10X)



Site E: Mouse 8

research; 1 mm; Form 24H-70% ETOH; 1 block

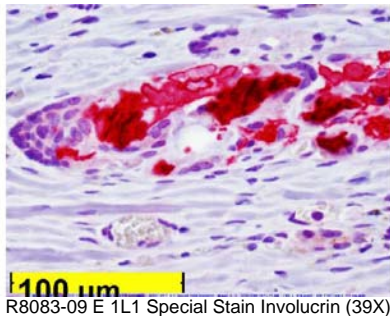
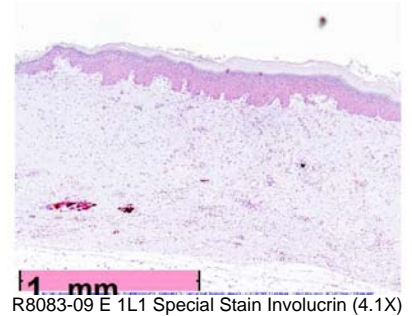
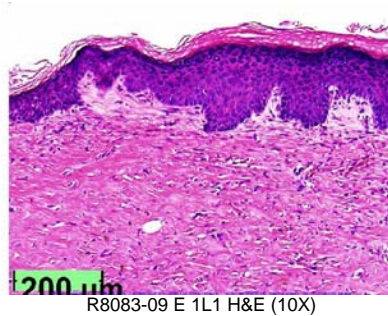
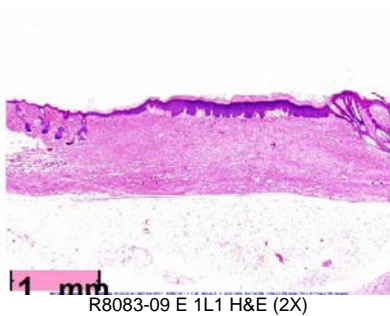
(ICD9: 795.4)

Clinical Impression: April 20, 2009

930MH+

Microscopic: Healing wound with hyperplastic epidermis exhibiting NO marking with polyclonal human involucrin antibody. Dermal granulomas, however, contain cornified cells, staining with 'human' involucrin antibody.

DIAGNOSIS: EPIDERMIS WITHOUT HUMAN CELLS (CONFIRMED NEGATIVE WITH 'HUMAN' INVOLUCRIN ANTIBODY)
SCAR
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)



Site F: Mouse 9

research; 1 mm; Form 24H-70% ETOH; 1 block

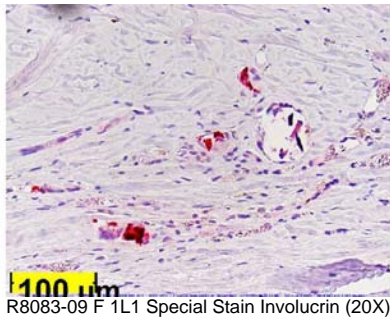
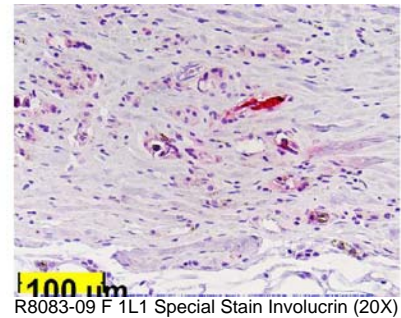
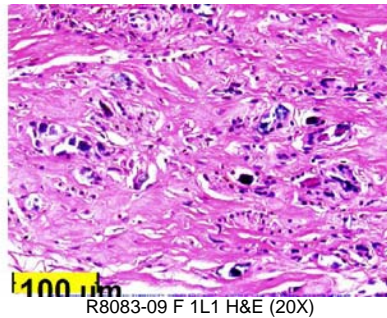
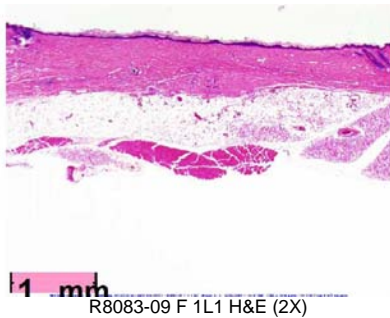
(ICD9: 795.4)

Clinical Impression: April 20, 2009

930MH+

Microscopic: Healing wound with hyperplastic epidermis exhibiting NO marking with polyclonal human involucrin antibody. Dermal granulomas, however, contain cornified cells, staining with 'human' involucrin antibody.

DIAGNOSIS: EPIDERMIS WITHOUT HUMAN CELLS (CONFIRMED NEGATIVE WITH 'HUMAN' INVOLUCRIN ANTIBODY)
SCAR
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)



Site G: Mouse 12

research; 1 mm; Form 24H-70% ETOH; 1 block

(ICD9: 795.4)

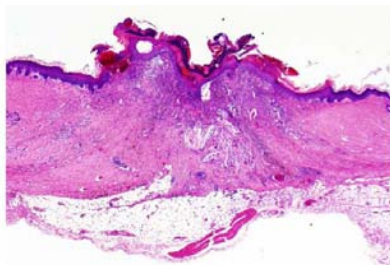
Clinical Impression: April 20, 2009

930MH+-DI
CT

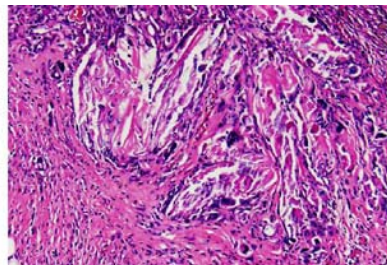
Microscopic: Healing wound with hyperplastic epidermis exhibiting NO marking with polyclonal human involucrin antibody. Dermal granulomas around layered cornified cells, staining with 'human' involucrin antibody.

DIAGNOSIS: EPIDERMIS WITHOUT HUMAN CELLS (CONFIRMED NEGATIVE WITH 'HUMAN' INVOLUCRIN ANTIBODY)
SCAR
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)

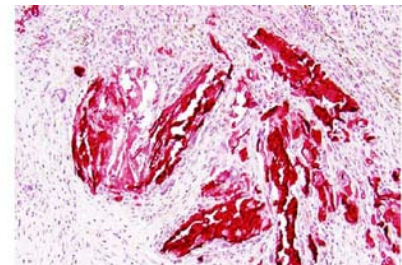
Note: The changes could fit with small cyst rupture



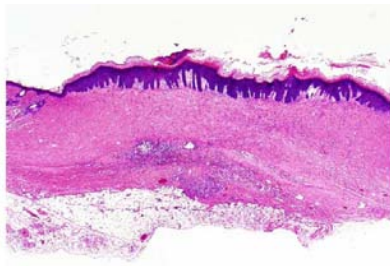
1 mm
R8083-09 G 1L1 H&E (2X)



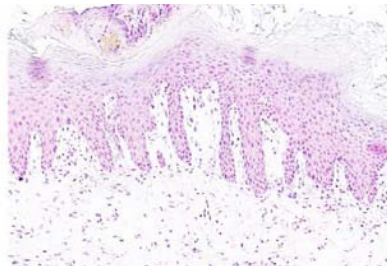
200 μm
R8083-09 G 1L1 H&E (9.8X)



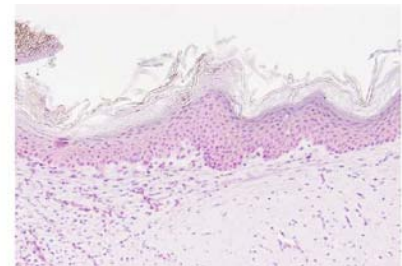
200 μm
R8083-09 G 1L1 Special Stain Involucrin (9.8X)



1 mm
R8083-09 G 1L1 H&E (2X)



200 μm
R8083-09 G 1L1 Special Stain Involucrin (9.9X)



200 μm
R8083-09 G 1L1 Special Stain Involucrin (9.9X)



Site H: Mouse 13

research; 1 mm; Form 24H-70% ETOH; 1 block

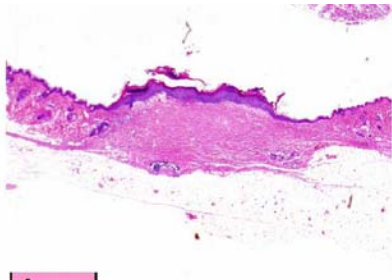
(ICD9: 795.4)

Clinical Impression: April 20, 2009

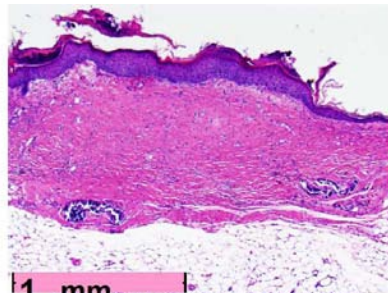
930MH+

Microscopic: Healing wound with hyperplastic epidermis exhibiting NO marking with polyclonal human involucrin antibody. Dermal granulomas, however, contain cornified cells, staining with 'human' involucrin antibody.

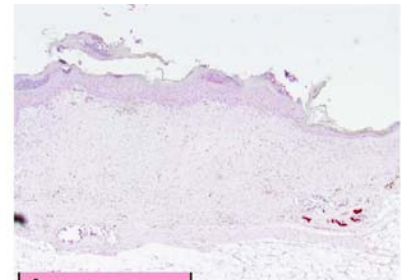
DIAGNOSIS: EPIDERMIS WITHOUT HUMAN CELLS (CONFIRMED NEGATIVE WITH 'HUMAN' INVOLUCRIN ANTIBODY)
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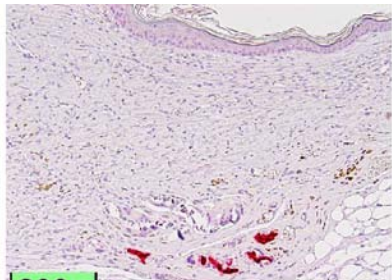
R8083-09 H 1L1 H&E (2X)



R8083-09 H 1L1 H&E (3.9X)



R8083-09 H 1L1 Special Stain Involucrin (3.9X)



R8083-09 H 1L1 Special Stain Involucrin (9.8X)



Site I: Mouse 14

research; 1 mm; Form 24H-70% ETOH; 1 block

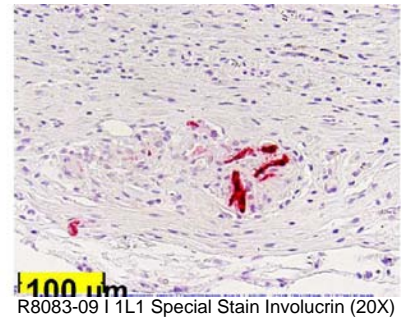
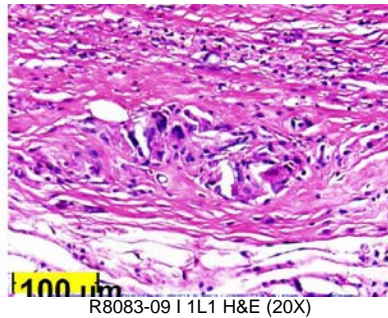
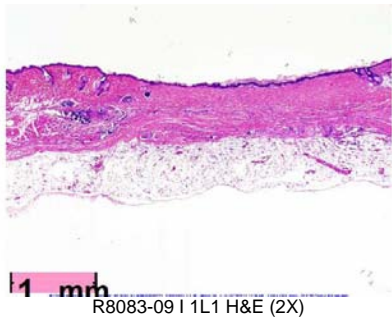
(ICD9: 795.4)

Clinical Impression: April 20, 2009

930MH+

Microscopic: Healing wound with hyperplastic epidermis exhibiting NO marking with polyclonal human involucrin antibody. Dermal granulomas, however, contain cornified cells, staining with 'human' involucrin antibody.

DIAGNOSIS: EPIDERMIS WITHOUT HUMAN CELLS (CONFIRMED NEGATIVE WITH 'HUMAN' INVOLUCRIN ANTIBODY)
SCAR
CORNIFIED CELL DERMAL GRANULOMAS (STAINING WITH 'HUMAN' INVOLUCRIN ANTIBODY)



Steve A. McClain MD

Steve A. McClain, M.D.
Electronically signed

this report includes
illustrative color images



ACKNOWLEDGEMENTS

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Jean Rooney, L.V.T, Senior Veterinary Technician
Nichole Steinhaufl, L.V.T., Veterinary Technician

Dermatopathologist and staff of McClain Laboratories

Dr. Steve McClain, M.D.
Joel Israel, Director of Research at McClain Laboratories

Living Skin Bank staff:

Dr. Jay G. Gao, Ph.D.
Alice Shih

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