

## Repair and reduction of photo damage with the dermaDNA™ MD testing and treatment kit

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### Summary

The single center, prospective controlled study evaluated the efficacy of DermaDNA™ MD kit (1 and 2) containing DNA-based (molecular) tests and skin treatment products for the detection and treatment of skin damage. N= 22 subjects with aging signs of the face were included in the 60 day study. Subjects gave informed consent and fulfilled the inclusion criteria prior to recruitment to the study. One subject discontinued the study on her own request and one subject was lost to follow up. N = 20 subjects completed the study (n = 20/20 female), all were over 40-years of age, (range 44 - 69-years), with a mean age of 54,90-years (SD 8,21). Of the included subjects 35% (n=7/20) had skin type<sup>1</sup> I, 45% (n=9/20) had skin type II and 20% (n=4/20) had skin type III. Skin type measured by saliva swabs (Lab MC 1R) showed that 25% (n=5/20) had skin type 2 and 75% (n=15/20) of subjects had skin type 3+ . N=9/20 (45%) subjects received kit 1 (SPF 30) for their study treatment and n=11/20 (55%) received kit 2 (SPF 45). Subjects noted their first wrinkles when they had a mean age of 39,06 - years (SD 11,68).

Regarding skin condition improvement during the study when comparing baseline (TX 0) and end of study (TX 4) nose swab results, 55% (n = 11/20) demonstrated an improvement ( $t(19) = -1.908$ ,  $p < 0.072$  (ns) mean - 0.80 (SD 1.87). The improvement demonstrated (for n = 11/20) was statistically significant ( $t(10) = - 4.38$ ,  $p < 0.01$ ) mean - 2.09 (SD 1.58). When evaluating the facial wrinkles with the 5-point Rao-Goldman<sup>2</sup> facial wrinkle scale at baseline and at the end of the study, the paired sample test (  $t(19) = 2.651$  mean 0.450 (SD 0.76) showed that the difference was statistically significant (  $p < 0.016$ ).

When the subjects were asked about their opinion on the result of treatment with the DermaDNA™ kit, 75% (n=12/20) noted a significant improvement of their skin condition and a reduction of their wrinkles, 10% (n=2/20) gave a neutral answer, 15% did not note an improvement n=3/20). Of the included subjects 85% (n=17/20 mean 4.20 (SD1.00) reported the tests to be user friendly and 90% (n=18/20) reported to have a clear understanding of the skin analysis and treatment kit use. 100% Of subjects reported the use of the tests and treatment products to be easy and 90% (n=18/20) reported to experience no discomfort when taking the saliva and nose swabs.

It was concluded that the evaluated tests gave a more detailed insight in the skin type and risk as well as level of skin damage. The prevention and treatment measures provided in the tested kits were shown to be beneficial in reducing photodamage.

<sup>1</sup> According to Fitzpatrick scale. Fitzpatrick et al., 1976. Pathak et al., 1980

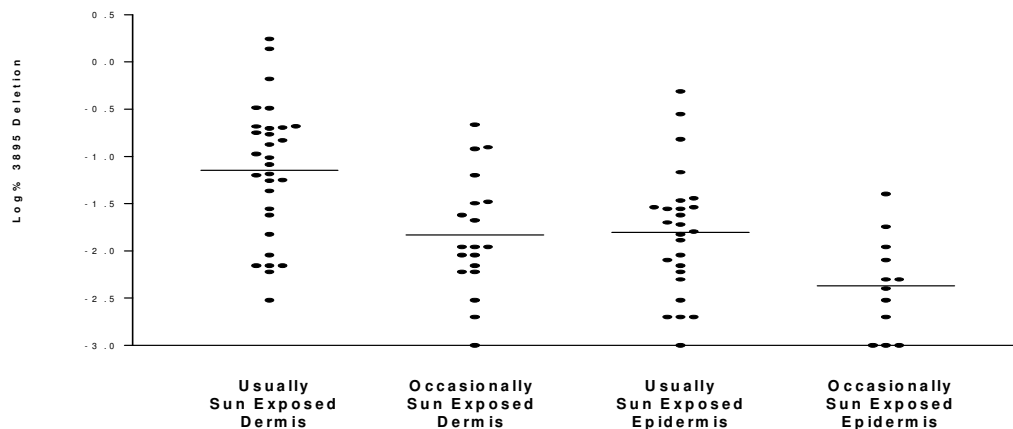
<sup>2</sup> Rao-Goldman 5-point facial wrinkle scale. Reference: Cosmetic Dermatology 2004, 17, 705-713.

## Introduction

The accumulation of mitochondrial DNA (mtDNA) mutations has been proposed as an underlying cause of the aging process. Oxidative stress is a mechanism thought to be involved in generating such mutations. Skin is frequently exposed to a potent mutagen in the form of ultraviolet (UV) radiation and mtDNA deletion mutations have previously been shown to accumulate with photoaging.<sup>3</sup>

The use of mtDNA damage as a biomarker of cumulative sunlight exposure in human skin is a relatively new field of research. Previous investigations have simply compared the frequency of occurrence of the mtDNA common deletion (CD), and to a much lesser extent that of tandem duplications (TDs), to distinguish between sun-protected and sun-exposed skin.<sup>4</sup>

Research<sup>5</sup> indicates the link between UVR exposure and DNA damage<sup>6</sup>. The DermaDNA™ MD test can accurately assess the skin DNA damage<sup>7</sup> caused by UVR. See figure 1, towers of damage.



**Figure 1: Towers of Damage: Level of mitochondrial DNA damage caused by ultraviolet radiation (UVR) from an epidermis skin sample taken from the Nose**

<sup>3</sup> Birket MJ, Birch-Machin MA. *Aging Cell*. 2007 Aug;6(4):557-64. Epub 2007 Jun 18

<sup>4</sup> Krishnan KJ, Birch-Machin MA. *J Invest Dermatol*. 2006 Feb;126(2):408-15

<sup>5</sup> Birch-Machin MA. *Mitochondria and Skin Disease*. *Clin Exp Dermatol* 2000; 25; 141-146

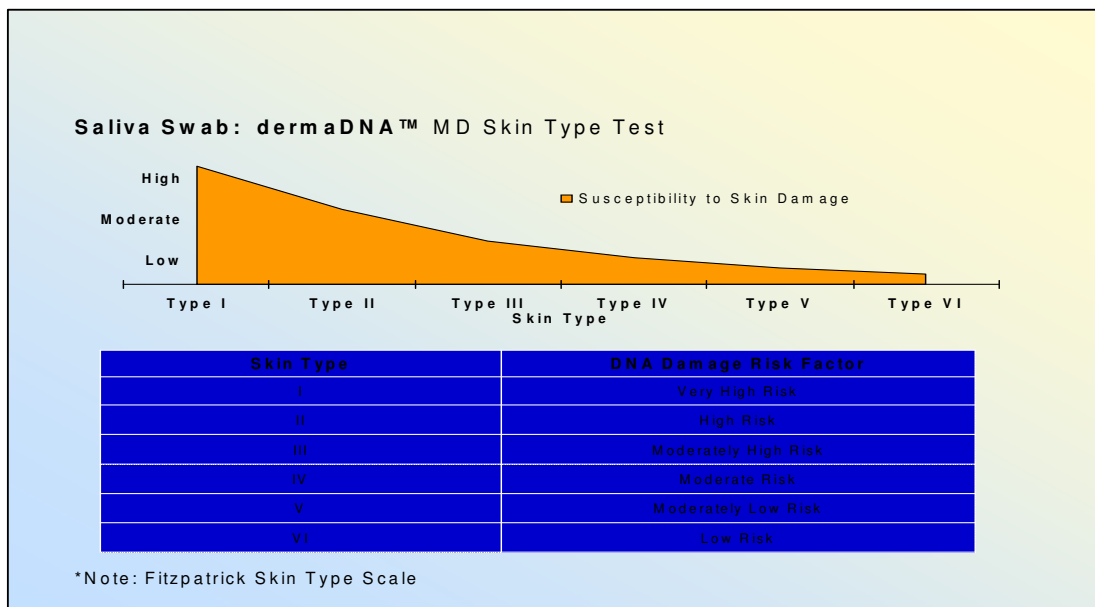
<sup>6</sup> Harbottle A, Krishnan KJ, Birch-Machin MA. Implications of using the ND1 gene as a control region for real-time PCR analysis of Mitochondrial DNA deletions in human skin. *JID* (2004) **122**, 1518–1521

<sup>7</sup> Krishnan KJ, Lindsey J, Lusher M, Lowes S & Birch-Machin MA. Current pitfalls in the measurement of the 4977 bp mitochondrial DNA common deletion in human skin. *J Invest Dermatol* (2003) **120**: 981–982.

Skin cell aging is accelerated by sun damage<sup>8</sup>, where DNA is the first structure of the cell to undergo aging. The subject's DNA Skin Type and sun lifestyle are critical contributors to the level of cell damage. Early detection is required to assess risk factors of UVR exposure and DNA damage, looking at both primary and secondary indicators. Primary indicators for early detection of skin damage are mtDNA damage and nuclear DNA mutations. Secondary indicators that enable late detection of skin damage are:

- Redness of Skin (erythema)
- Swelling of Skin (oedema)
- Pain and blistering of skin
- Peeling of Skin

The Damage Spectrum may show an increase in tanning, redness, time of exposure and enhanced Skin DNA damage. The science<sup>9</sup> and technology behind the test have been published in peer-reviewed scientific journals.<sup>10</sup>



**Figure 2: DermaDNA™ MD Skin Type Test and test of the level of damage**

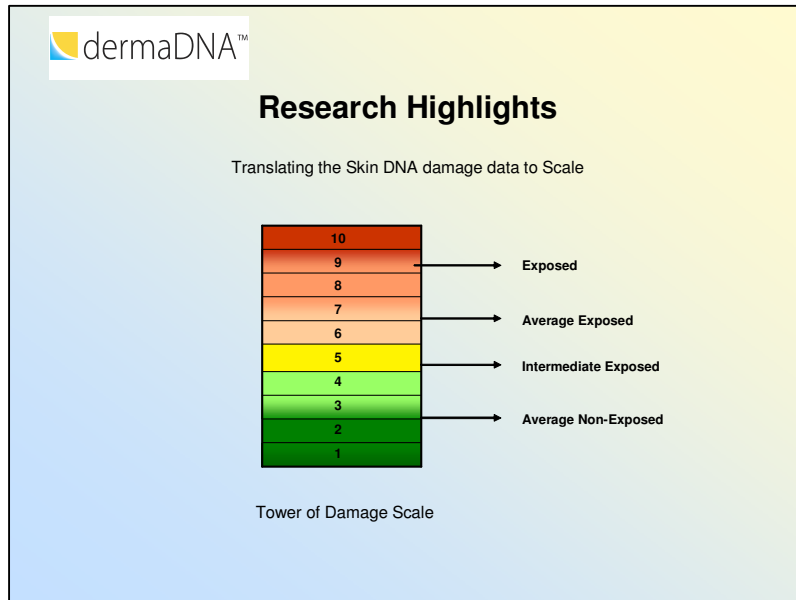
<sup>8</sup> Durham SE, Krishnan KJ, Betts J & Birch-Machin MA. Mitochondrial DNA damage in non-melanoma skin cancer. *Br J Cancer* (2003) **88**: 90–95.

<sup>9</sup> Birch-Machin MA, Tindall M, Turner R, Haldane F & Rees JL. Mitochondrial DNA deletions in human skin reflect photo-rather than chronologic aging. *J Invest Dermatol* (1998) **110**: 149–152.

<sup>10</sup> Ray AJ, Turner R, Nikaido O, Rees JL & Birch-Machin MA. The spectrum of mitochondrial DNA deletions is a ubiquitous marker of ultraviolet radiation exposure in human skin. *J Invest Dermatol* (2000) **115**: 674–679.

DermaDNA™ MD Skin Type Test determines the patient's susceptibility to skin damage and photo-aging through a saliva swab and determines DNA damage at the epidermis level through a nose skin swab, see figure 2.

The results of the tests is communicated with the clinical investigator accompanied by the skin care kit to repair and protect the skin from damage. The extent of damage is indicated on the tower of damage scale, see figure 3.



**Figure 3: Translating skin DNA data to a scale, presenting the tower of damage scale**

For the laboratory data of the present clinical study the actual CT values (from the DNA assay performed with the nose swab samples) were used. This information is more specific than the information on level of damage measured by a 10 point scale (tower of damage).

For example a level of damage of 1 is a CT value of 31-33. The CT values give a detailed score indicating the change in skin damage. See table 1.

The Fitzpatrick Classification Scale is typically used to classify a person's complexion and tolerance to sunlight. The Fitzpatrick scale classifies skin types from I to VI based on skin color, hair color and level of burning when exposed to sun. See table 2.

Examination of MC1R gene variants can classify skin into Fitzpatrick style 1-3+ skin types and hair colour. In addition there is evidence that MC1R gene variants are associated with higher risk for damage, independent of skin type and hair colour. See table 2.

## Materials and methods

There are two components to the DermaDNA™ MD test. The first component provides an analysis of skin type and a series of genetic risk factors (Fitzpatrick skin type score and Saliva Swab) and a lifestyle questionnaire. The second component determines DNA damage at the epidermis level. (Exposed Site = Nose Swab).

- DermaDNA™ MD kit 1 contains dermaDNA™ MD Gentle Foaming cleanser, dermaDNA™ MD Cellular Repair Serum, dermaDNA™ MD Daily Face Protect SPF 30 and dermaDNA™ MD Night Repair Cream.
- DermaDNA™ MD kit 2 contains dermaDNA™ MD Gentle Foaming cleanser, dermaDNA™ MD Advanced Cellular Repair Serum, dermaDNA™ MD Daily Face Protect SPF 45 and dermaDNA™ MD Night Repair Cream.

Scale	Zscore 1	Zscore 2	% of Pop. 1	% of Pop. 2	Overall group %	CT1	CT2	dCT
10	-4	-3	100	99.86	0.14	16.5	18.5	2
9	-3	-2	99.86	97.7	2.16	18.5	20.6	2.1
8	-2	-1	97.7	84	13.7	20.6	22.68	2.08
7	-1	-0.5	84	69.97	14.03	22.68	23.73	1.05
6	-0.5	0	69.97	49.8	20.17	23.73	24.77	1.04
5	0	0.5	49.8	30	19.8	24.77	25.82	1.05
4	0.5	1	30	15.7	14.3	25.82	26.85	1.03
3	1	2	15.7	2.2	13.5	26.85	28.92	2.07
2	2	3	2.2	0.2	2	28.92	31	2.08
1	3	4	0.2	0	0.2	31	33	2

**Table 1: Scoring system used for evaluation of the laboratory data**

The study evaluated the efficacy of a skin analysis and the two different skin treatment kits. The DNA-based (molecular) tests were evaluated looking at their efficacy to detect the risk for skin damage and actual skin damage that was present. Further the skin treatment products were tested for their efficacy of prevention and treatment of skin damage. The primary endpoint of the study was the change in the baseline tests and investigator evaluation scores per individual and per group compared with the final visit scores with regard to the following:

- I. Time to improvement of the signs expressing characteristics of aging skin on the face;
- II. Quality of skin condition;

Secondary endpoints included evaluations of the changes in baseline compared to final visit scores of the following:

- III. Ease of test and product use;
- IV. Safety of the treatment;

For this single center, prospective controlled study, subjects were selected according to the inclusion and exclusion criteria. After giving informed consent, they were interviewed after which a swab was taken from the skin of the nose and saliva. The swabs were sent for analysis to the laboratory. After the analysis was performed, material taken for testing was then destroyed and no records were kept at the laboratory. Details on the tests were noted as a pseudonym and could only be traced to the subject by the clinical investigators. The investigators guaranteed confidentiality of data and test materials obtained for the study at any point and time.

The results of the swabs were communicated with the clinician and the subject, informing where they were on the tower of damage. See figure 1,2 and 3 and table 1. The subjects were then informed by the clinician on the treatment and were given the applicable skin treatment kit; this was either Kit 1 (if the subject's results fell within levels 1 to 5 in the Tower of Damage) or Kit 2 (if the subject's results fell within levels 6 to 10 in the Tower of Damage Chart). For details see baseline data given in table 4.

<b>Visual Assessment</b>	<b>Fitzpatrick</b> I-VI Scale visual assessment	<b>MC1R</b> 1-3+ Scale - MC1R
Skin Type Assessment and UV Tolerance based on Skin Type and Hair Colour	Fair skin and red hair, characterized by low melanin content and a low eumelanin to pheomelanin ratio, is associated with poor tanning ability following sun exposure	Genetic examination of amino acid positions 84, 142, 151, 160, 294 are Fitzpatrick style assessments based on skin type and hair colour.
Independent Examination of Genetic Variants that indicate elevated risk for DNA Damage	N/A	MC1R genotype and responses of melanocytes to UVR. Examination of 142, 151, 160, 294. are strongly associated with red hair phenotype and reduced tanning ability.

**Table 2: Skin type assessment by Visual assessment, Fitzpatrick scale and MC1R test**

After the treatment was started, at day 14, 28 and 60 a nose swab was taken, the results were compared with the subjects' base line nose swab data. For the clinical evaluation DermaDNA test and treatment kit (Dkit) was used during a 60 day (8 week) treatment period. All the applicable tests and products were in the provided kit.

Daily cleansing of the face took place using the cleansing product provided in the kit. Then the serum was applied, after which the SPF product was applied. At night the face was cleansed with the cleanser provided, then the serum was applied followed by the night cream.

After recruitment to the study subjects filled out a sun lifestyle questionnaire. Before treatment was started, skin condition was measured with the Visia machine (Canfield Scientific) and physician assessment was carried out. Assessment and evaluation of skin condition was performed by the clinical investigator according to the time schedule described. For details see table 3.

	Screen <b>TX 0</b>	Day 0 <b>TX 1</b>	Day 14 <b>(+/-) TX 2</b>	Day 28 <b>(+/-) TX 3</b>	Day 60 <b>(+/-) TX 4</b>
Inclusion/Exclusion Criteria	x				
Written informed consent	x				
Medical history	x				
Pregnancy Test <sup>1</sup>	x				
Concomitant medications query	x	x	x	x	x
Adverse event query			x	x	x
Physician assessment		x	x	x	x
Visia		x		x	x
Testing saliva swab	x				
Testing nose swab	x		x	x	x
Patient fills out sunlife style questionnaire	x				
Treatment utilizing stated parameters		x	x	x	x
Subject Questionnaire	x				x
End of Study					x

**Table 3: Activities during the study and assessment schedule**

**Analyses plan:**

Table 3 provides information on the assessments, the number of visits and activities during these visits. The effectiveness of DermaDNA test and treatment kit (Dkit) was measured by the change in the wrinkle grading scales (both from the laboratory tests as well as the observational scale) used from the pre-treatment level. For the grading scales used see figure 3 and table 1.

Data was collected using a questionnaire. For study data management and analysis SPSS 16.0 (Windows)<sup>11</sup> statistical software was used.

Data management and analysis was performed by Dr. A. Andriessen and Drs. H. Andriessen (MSc) using where appropriate ANOVA, student t-test and Mann-Whitney test for N=20. Tests were carried out at the 5% significance level. The confidence interval was 95%.

Subject no	Vita-min	Freq. %	Anti – micro-bial	Freq. %	cleanser	Freq. %	Serum/cream	Freq. %
300	Vit D, Multivit	1 (5%)						
301							Mary K <sup>®</sup> blemish cover	1 (5%)
307			Topical Erythro-mycin	1 (5%)				
314					Dove <sup>®</sup> soap	1 (5%)	Lubriderm <sup>®</sup> lotion, Cetaphil <sup>®</sup> , Olay <sup>®</sup> regenerative	1 (5%) 1 (5%) 1 (5%)
315					Olay <sup>®</sup> cleanser	1 (5%)	Olay <sup>®</sup> regenerative	1 (5%)
321							Este lauder <sup>®</sup> serum	1 (5%)
<b>total</b>		<b>1 (5%)</b>		<b>1 (5%)</b>		<b>2 (10%)</b>		<b>6 (30%)</b>

**Table 4: Topical and/or facial skin condition related treatment before the study was started**

<sup>11</sup> Raynald Levesque, SPSS Programming and Data Management: A Guide for SPSS and SAS Users, Fourth Edition (2007), SPSS Inc., Chicago Ill.

## Results

N= 22 subjects with aging signs of the face were included in the study. Subjects fulfilled the inclusion criteria prior to recruitment to the study. One subject discontinued the study on her own request and one subject was lost to follow up.

N = 20 subjects completed the study (n = 20/20 female), all were over 40-years of age, (range 44 - 69-years), with a mean age of 54,90-years (SD 8,21). Of the subjects 45% was menopausal, 25% had a medical history of depression, and 20% had a thyroid related disease. Topical and/or facial skin condition related treatment was used by 30% (n = 6/20) of subjects before the start of the study, this treatment was discontinued where relevant for the study. For details see table 4.

Of the included subjects 35% (n=7/20) had skin type<sup>12</sup> I, 45% (n=9/20) had skin type II and 20% (n=4/20) had skin type III. For the included subjects, skin type measured by saliva swabs (Lab MC 1R) 25% (n=5/20) had skin type 2 and 75% (n=15/20) of subjects had skin type 3+ . For details on determining skin type see also figure 2. For baseline details see fig. 5.

N=9/20 (45%) subjects received kit 1 (SPF 30) for their study treatment and n=11/20 (55%) received kit 2 (SPF 45). Subjects noted their first wrinkles when they had a mean age of 39,06 - years (SD 11,68).

The evaluation of the sun life style questionnaire showed that 10% (n=2/20) of the subjects exposed their face to a sun bed over the past 6 months prior to recruitment to the study. None of the subjects used a sun bed for a shorter period than six months prior to the start of the study.

Six months prior to the start of the study 30 % (n=6/20) of the subjects went for a sun destination vacation, 35% (n=7/20) visited a sun destination over the past 3 months, 5% (n=1/20) over the past month and 5 % (n=1/20) went to a sun destination over the past two weeks. Of the included subjects 50% (n=10/20) used daily sun screen on their faces prior to study recruitment and 30 % (n=6/20) reported that they like to expose their skin to the sun.

At baseline (TX 0- screening, for baseline details see also table 5) the level of damage assessed by using a swab of the epidermis at the side of the nose (laboratory test) showed that 10% (n=2/20) had level 2 (average, non exposed), 30% (n=6/20) had level 3 (average non exposed), 35% (n=7/20) had level 4 (intermediary exposed), 15% (n=3/20) had level 5 (intermediary exposed, 5% (n=1/20) had level 6 (average exposed) and 5% (n=1/20) had level 7 (average exposed). See also figure 2 and 3 for information on the tower of damage and reading of the laboratory results.

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<sup>12</sup> According to Fitzpatrick scale. Fitzpatrick et al., 1976. Pathak et al., 1980

Subject number	TX 0 Fitzpatr. Skin Type (observation)	TX 0 MCIR skin type saliva (Lab)	TX 0 Level of damage nose (lab)	TX0 CT (lab)	TX 0 Rao-Wrinkle Scale	Kit
300	3	3+	3	28.10	4	CR SPF 30
301	2	3+	4	25.15	2	Adv. CR SPF 45
302	1	3+	3	27.17	5	Adv. CR SPF 45
303	2	3+	6	22.81	4	Adv. CR SPF 45
304	1	3+	4	25.51	3	Adv. CR SPF 45
305	1	3+	4	27.43	4	CR SPF 30
307	1	2	2	29.44	5	Adv. CR SPF 45
308	1	3+	4	24.95	4	CR SPF 30
309	2	2	4	25.02	3	Adv. CR SPF 45
310	2	3+	5	24.46	5	Adv. CR SPF 45
311	1	2	7	22.23	3	Adv. CR SPF 45
312	2	3+	5	24.55	3	CR SPF 30
313	1	3+	5	24.53	4	CR SPF 30
314	2	2	3	26.10	4	Adv. CR SPF 45
315	3	3+	4	25.17	2	CR SPF 30
316	1	2	4	25.74	3	Adv. CR SPF 45
317	2	3+	4	25.58	4	Adv. CR SPF 45
319	3	3+	3	25.85	4	CR SPF 30
320	3	3+	3	27.14	3	CR SPF 30
321	2	3+	2	28.25	3	CR SPF 30

**Table 5: Baseline data, skin type, skin damage and treatment kit recommendation**

**Notes:**

- CR SPF 30 = Cellular Repair, (kit I)
- Adv. CR SPF 45 = Advanced Cellular Repair, SPF 45 9 (kit II)
- Subject no. 306 and 318 were withdrawn. Withdrawal was not study product related

N=20	Strongly disagree	disagree	neutral	agree	Strongly agree	mean	SD
The saliva test was easily performed	1/20 (5%)	0/20	0/20	5/20 (25%)	14/20 (70%)	4.55	.95
The saliva test did not give me discomfort	1/20 (5%)	0/20	0/20	5/20 (25%)	13/20 (65%)	4.35	1.22
The nose swab tests were easily performed	0/20	0/20	0/20	5/20 (25%)	15/20 (75%)	4.75	0.44
The nose swab tests did not give me discomfort	2/20 (10%)	0/20	0/20	5/20 (25%)	13/20 (65%)	4.35	1.22
The test gives clear descriptions of my skin type regarding aging signs	1/20 (5%)	3/20 (15%)	2/20 (10%)	8/20 (40%)	6/20 (30%)	3.75	1.21
The test helped to decide whether DermaDNA is right for skin type	2/20 (10%)	1/20 (5%)	5/20 (25%)	8/20 (40%)	4/20 (20%)	3.55	1.19
General impression of the DermaDNA™ kit is	1/20 (5%)	0/20	3/20 (15%)	7/20 (35%)	9/12 (45%)	4.15	1.04
Satisfaction with the current treatment for my skin	2/20 (10%)	2/20 (10%)	3/20 (15%)	10/20 (50%)	3/20 (15%)	3.50	1,19
DermaDNA™ kit improved my facial skin condition	2/20 (10%)	1/20 (5%)	2/20 (10%)	9/20 (45%)	6/20 (30%)	3,80	1.24
The information for the use of the test was sufficient and easy to understand	0/20	1/20 (5%)	3/20 (15%)	9/20 (45%)	7/12 (35%)	4.10	,85
Confidence about test results to determine skin type	1/20 (5%)	0/20	3/20 (15%)	11/20 (55%)	5/20 (25%)	3.95	,95
The tests are user friendly	1/20 (5%)	0/20	2/20 (10%)	8/20 (40%)	9/20 (45%)	4.20	1.00
General feeling on use of the test is	0/20	1/20 (5%)	3/20 (15%)	8/20 (40%)	8/20 (40%)	4,15	0.88

**Table 6: Subject's opinion on the skin analysis and outcome of treatment**

N=20	Strongly disagree	disagree	neutral	agree	Strongly agree	mean	SD
Categorization of understanding of the skin analysis and treatment kit?	0/20	0/20	6/20 (30%)	9/20 (45%)	5/20 (25%)	3.95	,76
Understanding the warnings in the information given	0/20	0/20	2/20 (10%)	9/20 (45%)	9/20 (45%)	4.35	0.67
Understanding the precautions in the information given	0/20	0/20	2/20 (10%)	9/20 (45%)	9/20 (45%)	4,35	0.67
Confidence use of kit correct and safely without supervision by a doctor.	0/20	0/20	1/20 (5%)	9/20 (45%)	10/20 (50%)	4.45	0.61
Use of the Derma DNA skin analysis and treatment kit is easy	0/20	0/20	1/20 (5%)	9/20 (45%)	10/20 (50%)	4,45	0.61
Test outcome will support in the choice of treatment	0/20	0/20	3/20 (15%)	11/20 (55%)	6/20 (30%)	4,15	,67
Expectation use of analysis and treatment kit is medically effective and safe	0/20	0/20	2/20 (10%)	11/20 (55%)	7/12 (35%)	4.25	,64
Satisfaction with the way the tests were taken and the information they give	0/20	0/20	3/20 (15%)	9/20 (45%)	8/20 (40%)	4,25	,72
The result of treatment with DermaDNA™ is better then the last treatment received	2/20 (10%)	0/20	6/20 (30%)	8/20 (40%)	4/20 (20%)	3.60	1.14

**Table 6: Subject's opinion on the skin analysis and outcome of treatment cont.**

When measured with the 5-point Rao-Goldman<sup>13</sup> facial wrinkle scale at baseline (TX 0), 15% (n=3/20) of subjects had level 2 (shallow but visible wrinkles), 35% (n=7/20) had level 3 (moderately deep wrinkles), 35% (n=7/20) had level 4 (Deep wrinkles with well-defined edges) and 15% (n=3/20) had level 5 wrinkles (Very deep with redundant folds).

<sup>13</sup> Rao-Goldman 5-point facial wrinkle scale. Reference: Cosmetic Dermatology 2004, 17, 705–713.

Subject No.	MCIR Skin Type	TX0 Level of # damage	TX 0 D0 +Ct	TX2 D14 +Ct	TX3 D28 +Ct	TX4 D60 +Ct
300	3+	3	28.10	26.57	27.95	27.19
301	3+	4	25.15	26.31	24.75	25.66
302	3+	3	27.17	25.15	27.42	26.69
303	3+	6	22.81	26.18	22.50	24.66
304	3+	4	25.51	25.10	25.96	23.99
305	3+	3	27.43	27.20	24.32	26.07
307	2	2	29.44	26.79	25.90	28.72
308	3+	4	24.95	27.18	25.00	24.85
309	2	4	25.02	25.50	23.95	25.95
310	3+	5	24.46	26.76	25.64	27.01
311	2	7	22.23	25.30	26.21	27.99
312	3+	5	24.55	26.03	25.44	26.97
313	3+	5	24.53	26.35	26.59	27.61
314	2	3	26.10	24.45	25.48	25.47
315	3+	4	25.17	23.79	24.95	27.78
316	2	4	25.74	27.78	25.06	26.43
317	3+	4	25.58	25.26	24.74	25.71
319	3+	3	25.85	27.27	28.93	28.26
320	3+	3	27.14	25.75	27.10	26.71
321	3+	2	28.25	24.65	29.87	27.46

**Table 7: Skin type, level of damage, Ct value (DNA assay of the nose swabs) and % change per individual during the study**

**Notes:**

- \* For the % of change, Inverse Up indicates less damage
- #Level of damage is defined according to the +Ct values (translated to the tower of damage) for details see figure 3 and table 1. For data analysis of the nose swab's taken during the study, the actual Ct values (from the DNA assay) were used. Rather than the tower of damage with the 10 point scale, this information is more refined. For example a level of damage of 1 is a Ct value of 31-33. The Ct value gives a detailed score more clearly indicating the change in skin condition.

Skin properties improvement, % change ( scale 0 - 4*)	mean	SD
Change in Small Wrinkles and Fine Lines (RAO) <sup>14</sup>	1,10	0,91
Change in Hyper-pigmentation <sup>15</sup>	0,75	0,72
Erythema <sup>16</sup>	0,95	0,83
Tenderness <sup>17</sup>	0,00	0,00
Skin Texture	1,00	0,70
facial lines or wrinkles around the lip area	0,95	0,76
Pore Size	0,90	0,79
Overall Appearance of the Skin <sup>18</sup>	0,95	0,61

**Table 8 : Skin properties % of improvement\* when comparing baseline to end of the study**

**Note:**

- \*No Change (0%), 1 = Slight Change (1-25%), 2 = Minimal Change (26-50%), 3 = Moderate Change (51-74%), 4 = Significant Change (75-100%)

<sup>14</sup> Rao-Goldman 5 point wrinkle scale (1 = not visible, 2 = shallow but visible, 3 = moderately deep, 4 = deep, well defined, 5 = very deep with redundant folds)

<sup>15</sup> Overall Appearance of the Skin according to the global severity score (4-point scale). 0 = No sun damage present (there is no erythema present on the subject's face), 1 = Mild amount of sun damage present (erythema), 2 = Moderate amount of sun damage present (erythema involving at least 1/3 of face), 3 = Severe amount of sun damage present (erythema involving at least 1/2 of face).

<sup>16</sup> Erythema grade (5-point) 0 to 4 scale (0 = none, 1) minimal, scant rare erythema, 2) mild, easily seen erythema involving up to 1/3 of the face, 3) moderate, easily seen erythema involving between 1/3 to 2/3 of the face, 4) severe, easily seen erythema involving over 2/3 of the face).

<sup>17</sup> Tenderness of the facial skin (4-point scale) 0 to 3 (0) no burning/stinging, 1) minimal burning/stinging, 2) moderate burning/stinging, 3) severe burning/stinging.

<sup>18</sup> Overall Appearance of the Skin according to the global severity score (4-point scale). 0 = No sun damage present (there is no hyperpigmentation or erythema present on the subject's face), 1 = Mild amount of sun damage present (slight hyperpigmentation and erythema), 2 = Moderate amount of sun damage present (hyperpigmentation and erythema involving at least 1/3 of face), 3 = Severe amount of sun damage present (hyperpigmentation and erythema involving at least 1/2 of face).

A subject questionnaire was filled out at baseline and at the end of the study ( for details see table 6). Of the subjects 60% (n=13/20 mean 3.55 (SD 1.19) felt that the tests helped them to decide whether DermaDNA is right for their skin type. 80% Of subjects (n=16/20 mean 3.95 (SD 0.95) expressed to have confidence that the test results reflect their actual skin condition.

Of the included subjects 85% (n=17/20 mean 4.20 (SD1.00) reported the tests to be user friendly. 90% (n=18/20) Reported to have a clear understanding of the skin analysis and treatment kit use as well as the warnings and precautions given in the provided information and 10% gave a neutral answer. 100% Of subjects reported the use of the tests and treatment products to be easy and safe and 90% (n=18/20) reported to experience no discomfort when taking the saliva and nose swabs.

When the subjects were asked about their opinion on the result of treatment with the DermaDNA™ kit, 75% (n=12/20) noted a significant improvement of their skin condition and a reduction of their wrinkles, 10% (n=2/20) gave a neutral answer, 15% did not note an improvement n=3/20).

Rao-Goldman facial wrinkle scale N=20	TX 0	End TX 4
Level 2	15% (n=3/20)	25% (n=5/20)
Level 3	35% (n=7/20)	50% (n=10/20)
Level 4	35% (n=7/20)	20% (n=4/20)
Level 5	15% (n=3/20)	5% (n=2/20)
		<b>*p &lt; 0.016</b>

**Table 8: Rao-Goldman facial wrinkle scale N=20 comparing baseline and end of study evaluation**

**Note:** \* = statistically significant (the paired sample test ( t(19) = 2.651 mean 0.450 (SD 0.76)

Regarding skin condition improvement during the study when comparing baseline (TX 0) and end of study (TX 4) nose swab results (Ct value from the DNA assay (nose swab) when measured within the subjects, 55% (n = 11/20) demonstrated an improvement (t(19) = -1.908,  $p < 0.072$  (ns) mean - 0.80 (SD 1.87). The improvement demonstrated (for n = 11/20) was statistically significant (t(10) = - 4.38,  $p < 0.01$ ) mean - 2.09 (SD 1.58).

When assessing the wrinkle grade at baseline vs the end of the study (TX 4) measured within the individual 10% (n=2/20) showed a significant improvement and 60% (n= 12/20) had no change in the score. Table 8 gives details on the assessment of skin properties using various scales.

N=20	mean	SD	t- test	P value
<b><i>Skin moisture</i></b> (1 = very moist – 4 very dry)	,700	1,174	2,666	<b>,015*</b>
Oily skin (1= not - 4=very)	,150	,587	1,143	,267
<b><i>skin texture</i></b> 1 = smooth and soft, 2 = slightly coarse and grainy, 3 = very coarse and grainy, 4 = bumpy and uneven	,600	1,095	2,449	<b>,024*</b>
<b><i>Skin pores</i></b> (1 = invisible 5= many enlarged pores visible)	,450	,887	2,269	<b>,035*</b>
<b><i>facial lines or wrinkles around the eye area</i></b> 1 = none 2 = a few around eyes 3- a few around nose 4 = a few on cheek 5 = many all over	,200	1,436	,623	,541
<b><i>facial lines or wrinkles around the lip area</i></b> 1 = not visible, 2 = shallow, but visible, 3 = moderately deep, 4 = deep, 5 = very deep	,400	1,188	1,506	,148
<b><i>Cheek skin</i></b> 1 = very tight or firm, 2 = tight, 3 = little tight , 4 = loose, 5 = very loose and baggy	,400	,940	1,902	,072
<b><i>facial skin thickness</i></b> 1 = thick, 2 = normal, 3 = thin, 4 = very thin	,050	,510	,438	,666
<b><i>Are you pleased with the properties of your facial skin?</i></b> 1 = very pleased, 2 = mostly pleased, 3 = displeased, 4 = very displeased	,900	1,021	3,943	<b>,001*</b>

**Table 9: Subject's opinion on skin properties when comparing baseline (TX 0) vs end of the study (TX 4)**

- Marked with \* = statistically significant improvement, Paired Samples Tests were performed for baseline vs end results (95% Confidence Interval)

N = 20	Yes	No	Not sure	Mean (SD)
Improvement in your fine lines or wrinkles	11 (55%)	5 (25%)	4 (20%)	0.69 (SD 0.48)
Improvement skin elasticity or tightness	11 (55%)	7 (35%)	2 (10%)	0.61 (SD 0.50)
Do you notice any improvement in your <u>skin texture</u> (pore-size, roughness, touch, ) when using the products?	13 (65%)	6 (30%)	1 (5%)	0.68 (SD 0.48)
you notice any improvement in your <u>skin hydration</u> when using the products?	17 (85%)	2 (10%)	1 (5%)	0.89 (SD 0.32)
Do you notice any improvement in your global skin appearance (impurities, youthful shine, freshness, glow, tone, color, ...) when using the products?	12 (60%)	6 (30%)	2 (10%)	0.67 (SD 0.49)
Do you like the way the products feel on your skin?	18 (90%)	2 (10%)	0 (0%)	0.90 (SD 0.31)
Do you like the scent of the products?	16 (80%)	1 (5%)	3 (15%)	0.94 (SD 0.24)
Do you notice any differences in fine lines or wrinkles?	8 (40%)	7 (35%)	5 (25%)	0.53 (SD 0.52)

**Table 10: Subject's opinion on facial condition and product properties evaluated at the end of the study (TX 4)**

The results of the Visia (Canfield) assessments were in line with the results given in table 8.

When measured with the 5-point Rao-Goldman<sup>19</sup> facial wrinkle scale at baseline (TX 0), 15% (n=3/20) of subjects had level 2 (shallow but visible wrinkles), 35% (n=7/20) had level 3 (moderately deep wrinkles), 35% (n=7/20) had level 4 (Deep wrinkles with well-defined edges) and 15% (n=3/20) had level 5 wrinkles (Very deep with redundant folds).

<sup>19</sup> Rao-Goldman 5-point facial wrinkle scale. Reference: Cosmetic Dermatology 2004, 17, 705–713.

When measured with the 5-point Rao-Goldman facial wrinkle scale at the end of the study (TX 4), 25% (n=5/20) of subjects had level 2 (shallow but visible wrinkles), 50% (n=10/20) had level 3 (moderately deep wrinkles), 20% (n=4/20) had level 4 (Deep wrinkles with well-defined edges) and 5% (n=2/20) had level 5 wrinkles (Very deep with redundant folds).

When evaluating the facial wrinkles with the 5-point Rao-Goldman facial wrinkle scale at baseline and at the end of the study, within subjects, the paired sample test ( $t(19) = 2.651$  mean 0.450 (SD 0.76) showed that the difference was statistically significant ( $p < 0.016$ ).

### **Conclusion**

It was concluded that the evaluated tests give a more detailed insight in the skin type and risk as well as level of skin damage. This individual approach based on quantitative parameters to identify skin features and risk for skin damage will positively impact on physicians communication, subject awareness, concordance, and preventive measures.

The prevention and treatment measures provided in the tested kits were shown to be beneficial in reducing facial skin photodamage.