Milan, June 21th 2010

INSTRUMENTAL, CLINICAL and SUBJECTIVE EVALUATION of the EFFICACY of a COADJUTANT LOCAL TREATMENT for PREVENTING the HAIR LOSS by means of PHOTOTRICHOGRAM TECHNIQUE

METHOD: Ref. E29U

CUSTOMER: **SYMRISE Inc**
300 North Street
TETERBORO, New Jersey 07608, USA

PRODUCTS: **AT09073 A**

Ref.ISPE: 70/10/01 – 766/09

AT09073 B

Ref.ISPE: 70/10/02 – 767/09

STARTING DATE OF THE STUDY: 22/02/'10

COMPLETION DATE: 17/06/'10

ETHICAL AND QUALITY CRITERIA

The current study was carried out in compliance with the quality assurance system requirements, according to the principles of good laboratory practice (GLP) and good clinical practice (GCP), as well as the principles established by the World Medical Association in the Declaration of Helsinki.

REFERENCES

The data given in this report are exclusively related to the tested sample.
This report can be only in full reproduced.

ISPE s.r.l.
Director of the Laboratory
Dr. Luigi Rigano

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1. SAMPLES DATA SHEET

SAMPLES REF.: **AT09073 A**

Ref.ISPE: 70/10/01 – 766/09

AT09073 B

Ref.ISPE: 70/10/02 – 767/09

SAMPLES ARRIVAL DATE: 17/02/'10

PRODUCTS:

- PHYSICAL FORM: fluid
- COLOUR: pale yellow

QUALITATIVE FORMULAS:

- KNOWN / /
- OTHER INFORMATION / /

OTHER INFORMATION RELATED TO THE PRODUCTS SAFETY:
None.

FILE: 2 samples with the code number **Ref. ISPE 70/10/01 – 766/09** and **Ref. ISPE 70/10/02 – 767/09** and the study findings will be kept filed in our archives for two years and for ten years respectively. After these periods, the samples and the findings report will be discarded, unless otherwise required by the client.

INSTRUMENTAL, CLINICAL and SUBJECTIVE EVALUATION of the EFFICACY of a COADJUTANT LOCAL TREATMENT for PREVENTING the HAIR LOSS by means of PHOTOTRICHOGRAM TECHINIQUE (Ref. E29U)

2. AIM OF THE STUDY

Aim of the study is to evaluate the efficacy in preventing the hair loss of 2 local products.

The study is carried out as a double blind study on 24 volunteers suffering from androgenetic alopecia: II–III stage of Hamilton's scale for men and I-II stage of Ludwig's scale for women (see Appendix). 12 subjects apply product A and 12 product B.

The product efficacy is evaluated by means of the phototrichogram technique, performed at the beginning and after 45 days and 3 months of treatment.

In androgenic alopecia a progressive inversion of the normal anagen/telogen ratio due to the increase of the percentage of hair in rest phase is observed. The discovery of a statistically significant decrease of the number or the percentage of hair in telogen phase for the hair in anagen phase gives evidence of the efficacy of the treatment.

At each check a clinical assessment of the scalp is carried out to evaluate the presence of dandruff, seborrhoea, erythema, itching and burning together with the tolerability of the treatment.

Furthermore, at the end of the period of application, the volunteers express their opinion about the efficacy and the pleasantness of the treatment.

3. SELECTION OF THE VOLUNTEERS

3.a. Criteria for recruitment and admission

At the beginning of the study each volunteer signed the informed consent drawn up by the technicians. Two groups of 12 volunteers of both sexes (5 males and 7 females in each group, mean age 49.0 for A group and 44.4 years for B group) were included in this study according to the following criteria:

3.b. Inclusion criteria

- Race: Caucasian;
- Age: between 18 and 60 years;
- Sex: male and female;
- Health state: absence of disease during the period of the study and immediately preceding it;
- Contactability;
- Subjects suffering from androgenic alopecia: II-IV stage of Hamilton's scale for men and I-II stage of Ludwig's scale for women (see Appendix).

3.c. Exclusion criteria

- Subjects who are undergoing any topical or systemic therapy which could interfere with the results of the test;
- Pregnant and breast-feeding women;
- Subjects affected by skin diseases;
- Subjects with a history of intolerance to drugs and/or cosmetic products;
- Subjects who have previously undergone treatments for alopecia within the last three months.

3.d. Drop-out

The study can be interrupted due to the reasons mentioned below:
- subject's free choice;
- clinical reasons not related to the treatment (ex. illness, surgery, etc.);
- clinical reasons related to the treatment (irritating or allergical reactions).

All the drop-out cases appeared during this study are underlined.

3.e. Restrictions

For the whole duration of the study the subjects must not use anti hair loss treatments different from the tested product.

4. INSTRUMENTS

4.a. FotoFinder Dermoscope and Trichoscan Professional Ver.2.0

The phototrichogram consists of taking a photograph of a defined area of the scalp after hair clipping, and to repeat it after a certain period of time. The period of time should be long enough to permit the evaluation of the growth of a hair segment (48 hours). The growth is then evaluated by comparing the two pictures: hairs that have grown are in the anagen phase and those that have not are believed to be in the telogen phase.
The area is analysed by TrichoScan (Fotofinder Dermoscope and Trichoscan professional Ver. 2.0) a recently developed method based on the principle of the epiluminescence microscopy. A frontal glass slide mounted on the recording device reduces the curvature of the scalp and permits a better image definition. The contrast-enhanced phototrichogram is designed to help to distinguish hair on a skin background of the same colour. Application of dyes on hair is quite convenient for such a purpose.
The hair is clipped in an area of 1 cm^2. 48 hours later, the hair is dyed with a black hair colour. Then a digital image of 20-fold magnification (analyzed area 0.651 cm^2) is taken by means of a epiluminescence microscopy system.

The software analyzed the digital image and detected the hair density (n° hair/1 cm^2), the number and percentage of hair in anagen phase and the number and percentage of hair in telogen phase in the analyzed area.

4.b. Instruments for shaving

Moser shaver with spacer for pre-shaving in the direction of the hair growth.

Panasonic micro-shaver for shaving against the hair.

5. METHOD

5.a. Method of evaluation

The study was carried out on 24 volunteers, suffering from androgenic alopecia: II–IV stage of Hamilton's scale for men and I-II stage of Ludwig's scale for women (see Appendix).

The assignment of subject number and subsequent placement on the randomization chart was given in order of appearance at the study centre on the first day of the test.
12 subjects applied the **AT09073 A** product and the other 12 used the **AT09073 B** product on the scalp, 2 ml a day, for 3 months. The products were given to the subjects in anonymous containers with the following instructions:

- shake well the mixture, remove the top and put the pipette in the container, filling it till 2 ml;
- spread the product on the scalp (dry or wet hair);
- massage for 3-4 minutes without rinsing.

The volunteers were asked to wash their hair 48 hours before each check and not to apply styling products during the 48 hours preceding the visit.

The phototrichogram was performed at the beginning, after 45 days and at the end of the 3 months of treatment. This technique consists in taking a photograph of an area of 1 cm^2 of the scalp 48 hours after the hair shaving. It consents to evaluate all the phases of hair growth during the time.

5.b. Analysis of the phototrichograms

By means of the software **Trichoscan Professional**, the following parameters were calculated at each control:

- the **mean density** of hair per cm^2;

- the **percentage** and the **number** of **anagen** hair (phase of active growth) related to the area analyzed by the software (0.651 cm^2);

- the **percentage** and the **number** of **telogen** hair (rest phase) related to the area analyzed by the software (0.651 cm^2).

5.c. Objective dermatological evaluation

- A clinical observation of the scalp condition in order to assess the presence of dandruff, seborrhoea and the level of erythema was performed. The volunteers were asked to refer about possible itching and burning sensations perceived on the scalp.

A score was given according to a 4-point scale where:

0 = absent;
1 = mild;
2 = moderate;
3 = severe.

- Finally, the overall tolerability of the treatment was evaluated at the intermediate and at the final check. A score was given according to a 4-point scale where:

0 = poor tolerability;
1 = mild tolerability;
2 = moderate tolerability;
3 = very good tolerability.

5.d. Subjective evaluation

At the end of the test the volunteers filled in a questionnaire for the subjective evaluation of the efficacy and the pleasantness of the treatment.

For each question, the volunteers expressed their opinion on a 4-point scale where statements were related to four different perceived intensities.

Example: how do you judge the treatment as regards its efficacy in diminishing the hair loss?

- very effective
- fairly effective
- not very effective
- not effective at all

5.e. Mathematical Elaboration

Following the results of normality tests (Kolmogorov-Smirnov test, Lilliefors test and Shapiro-Wilk test) the data can be considered **parametric**.

Mean values and standard deviations were calculated for each parameter (density/cm^2, number of anagen and telogen hair) at all control times. The percentage of anagen and telogen hair is also reported.

Furthermore, the variation and the percentage of variation were calculated.

The data obtained at each check were compared by means of **Repeated Measures Analysis of Variance** and **Bonferroni Test**.
The comparison between the two products (active and placebo) was analyzed by means of **Bonferroni Test** for independent groups of data with more than 2 variables.

The differences between the groups of values were considered significant when the probability **p** was **≤ 0.05**.

Objective dermatological evaluation

Following the results of normality tests (Kolmogorov-Smirnov test, Lilliefors test and Shapiro-Wilk test) the data can be considered **non-parametric**.

Mean and standard deviation were calculated for each parameter at all the checks.
The data obtained at each check were compared by means of a non-parametric statistical test (**Friedman's Anova and Kendall's coefficient of concordance**).
The statistical comparison between the two treatments was performed by means of **Kruskall-Wallis ANOVA** for non-parametric independent data.

The differences between the groups of values were considered significant when the probability **p** was **≤ 0.05**.

Subjective evaluation

For each question the partial score was determined by summarising the answers given for each level of intensity.

This score has been reported as percentage score in the tables.

6. RESULTS: TABLES AND GRAPHS

6.a. ANALYSIS OF THE PHOTOTRICHOGRAM

HAIR DENSITY per cm^2:

AT09073 A: No statistically significant variation in the mean hair density per cm^2 was recorded.

T_0	T_{45days}	$T_{3months}$	Variation (%) $T_{45days} - T_0$	Variation (%) $T_{3months} - T_0$
mean 201.6 *std. dev. 62.4*	**mean 211.2** *std. dev. 61.9*	**mean 210.6** *std. dev. 68.1*	**9.6 (4.8 %)**	**9.0 (4.5 %)**
			p > 0.05	p > 0.05

AT09073 B: No statistically significant variation in the mean hair density per cm^2 was recorded.

T_0	T_{45days}	$T_{3months}$	Variation (%) $T_{45days} - T_0$	Variation (%) $T_{3months} - T_0$
mean 183.0 *std. dev. 41.5*	**mean 184.1** *std. dev. 42.7*	**mean 184.9** *std. dev. 38.0*	**1.1 (0.6 %)**	**1.9 (1.0 %)**
			p > 0.05	p > 0.05

No statistically significant difference was detected between the two products.

	Bonferroni test
($T_{45days} - T_0$) AT09073 A vs ($T_{45days} - T_0$) AT09073 B	p > 0.05
($T_{3months} - T_0$) AT09073 A vs ($T_{3months} - T_0$) AT09073 B	p > 0.05

NUMBER AND PERCENTAGE OF ANAGEN HAIR in the area analyzed by the software (0.651 cm^2):

AT09073 A: No statistically significant variation in the number of anagen hair was recorded.

N° anagen hair (%) T_0	N° anagen hair (%) T_{45days}	N° anagen hair (%) $T_{3months}$	Variation (%) $T_{45days} - T_0$	Variation (%) $T_{3months} - T_0$
mean 83.3 *std. dev. 27.0* **(67.6 %)**	**mean 86.6** *std. dev. 27.8* **(66.2 %)**	**mean 89.0** *std. dev. 33.7* **(68.3 %)**	**3.3 (4.0 %)**	**5.7 (6.8 %)**
			p > 0.05	p > 0.05

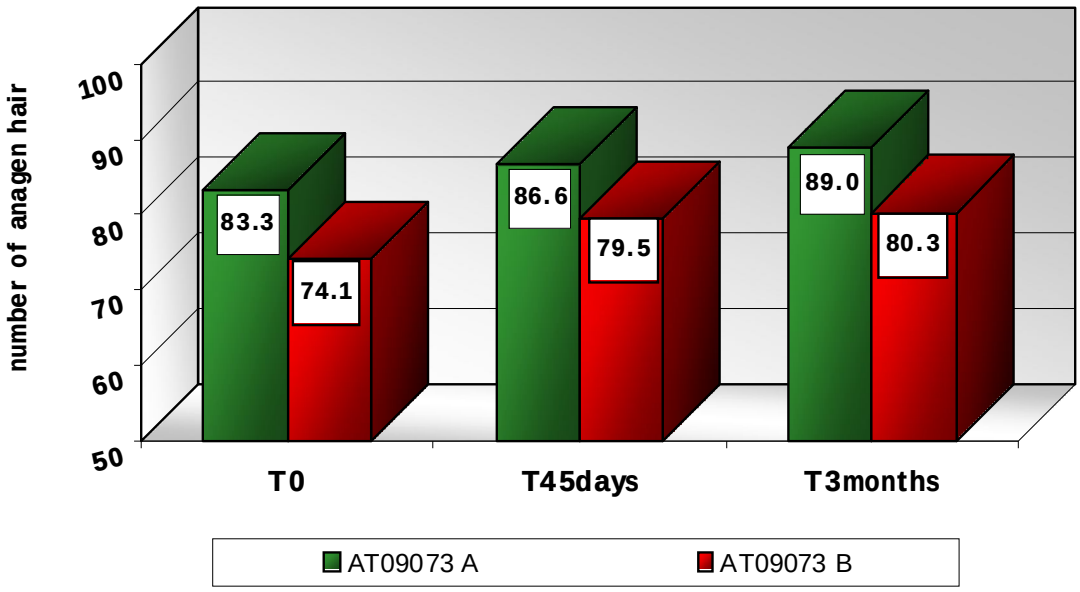
AT09073 B: No statistically significant variation in the number of anagen hair was recorded.

N° anagen hair (%) T_0	N° anagen hair (%) T_{45days}	N° anagen hair (%) $T_{3months}$	Variation (%) $T_{45days} - T_0$	Variation (%) $T_{3months} - T_0$
mean 74.1 *std. dev. 28.3* **(59.1 %)**	**mean 79.5** *std. dev. 26.7* **(64.2 %)**	**mean 80.3** *std. dev. 25.3* **(64.8 %)**	**5.4 (7.3 %)**	**6.2 (8.4 %)**
			p > 0.05	p > 0.05

No statistically significant difference was detected between the two products.

	Bonferroni test
($T_{45days} - T_0$) AT09073 A vs ($T_{45days} - T_0$) AT09073 B	p > 0.05
($T_{3months} - T_0$) AT09073 A vs ($T_{3months} - T_0$) AT09073 B	p > 0.05

Graph 1: Number of anagen hair recorded at each check for the 2 products.



NUMBER AND PERCENTAGE OF TELOGEN HAIR in the area analyzed by the software (0.651 cm^2):

AT09073 A: No statistically significant variation in the number of telogen hair was recorded.

N° telogen hair (%) T_0	N° telogen hair (%) T_{45days}	N° telogen hair (%) $T_{3months}$	Variation (%) $T_{45days} - T_0$	Variation (%) $T_{3months} - T_0$
mean 47.9 *std. dev. 45.8* **(32.4 %)**	**mean 50.9** *std. dev. 45.0* **(33.8 %)**	**mean 47.6** *std. dev. 46.2* **(31.4 %)**	**3.0 (6.3 %)**	**-0.3 (-0.6 %)**
			p > 0.05	p > 0.05

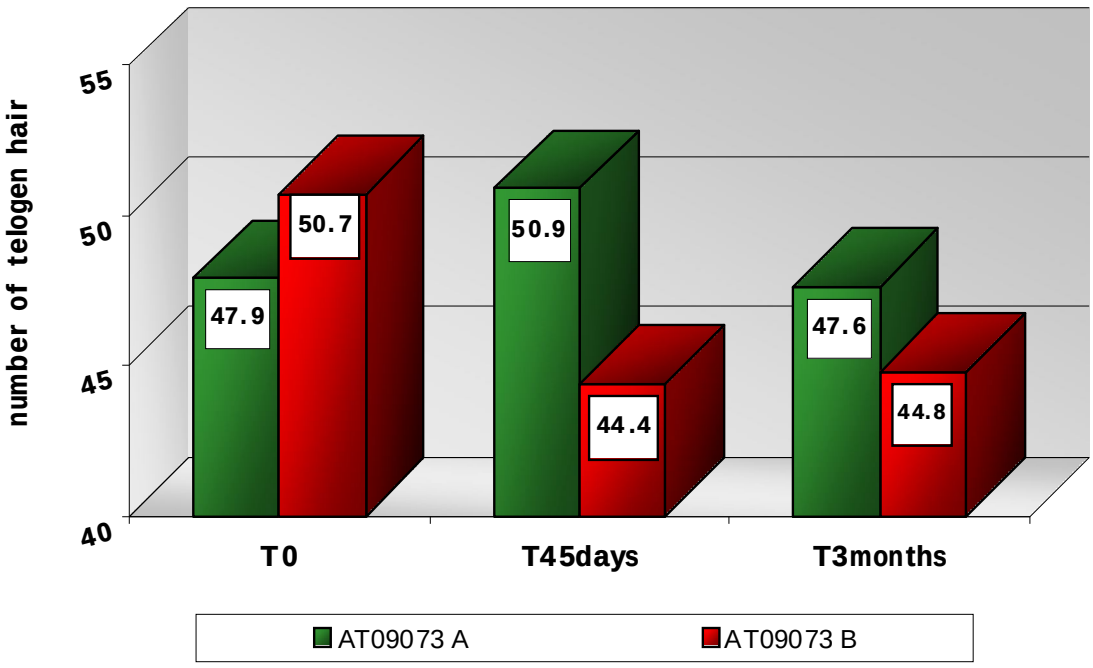
AT09073 B: No statistically significant variation in the number of telogen hair was recorded.

N° telogen hair (%) T_0	N° telogen hair (%) T_{45days}	N° telogen hair (%) $T_{3months}$	Variation (%) $T_{45days} - T_0$	Variation (%) $T_{3months} - T_0$
mean 50.7 *std. dev. 23.7* **(40.9 %)**	**mean 44.4** *std. dev. 24.3* **(35.8 %)**	**mean 44.8** *std. dev. 26.0* **(35.2 %)**	**-6.3 (-12.4 %)**	**-5.9 (-11.6 %)**
			p > 0.05	p > 0.05

No statistically significant difference was detected between the two products.

	Bonferroni test
($T_{45days} - T_0$) AT09073 A vs ($T_{45days} - T_0$) AT09073 B	p > 0.05
($T_{3months} - T_0$) AT09073 A vs ($T_{3months} - T_0$) AT09073 B	p > 0.05

Graph 2: Number of telogen hair recorded at each check for the 2 products.



6.b. OBJECTIVE DERMATOLOGICAL EVALUATION

A score was given according to a 4-point scale (*0 = absent; 1 = mild; 2 = moderate; 3 = severe*) for the presence/onset of dandruff, seborrhoea, erythema, itching and burning sensations.

A score was given according to a 4-point scale (*0 = poor; 1 = mild; 2 = moderate; 3= very good*) for the tolerability of the treatment.

AT09073 A	T_0	T_{45days}	$T_{3months}$	T_{45days} vs T_0	$T_{3months}$ vs T_0
DANDRUFF	**mean 0.4** *std. dev. 0.5*	**mean 0.9** *std. dev. 1.1*	**mean 0.4** *std. dev. 0.7*	p > 0.05	p > 0.05
SEBORRHOEA	**mean 1.1** *std. dev. 0.8*	**mean 0.8** *std. dev. 0.8*	**mean 0.7** *std. dev. 0.7*	**p < 0.05**	**p < 0.05**
ERYTHEMA	**mean 0** *std. dev. 0*	**mean 0** *std. dev. 0*	**mean 0** *std. dev. 0*	---	---
ITCHING	**mean 0.5** *std. dev. 0.9*	**mean 0.2** *std. dev. 0.6*	**mean 0.1** *std. dev. 0.3*	p > 0.05	p > 0.05
BURNING	**mean 0** *std. dev. 0*	**mean 0** *std. dev. 0*	**mean 0** *std. dev. 0*	---	---
TOLERABILITY	**- - -**	**mean 3.0** *std. dev. 0.0*	**mean 3.0** *std. dev. 0.0*	---	---

A statistically significant decrease in the level of seborrhoea was recorded at both control checks, while no statistically significant variation of dandruff, erythema, itching and burning sensation was detected during the period of treatment.
The product was very well tolerated.

AT09073 B	T_0	T_{45days}	$T_{3months}$	T_{45days} vs T_0	$T_{3months}$ vs T_0
DANDRUFF	**mean 0.8** *std. dev. 0.7*	**mean 0.4** *std. dev. 0.9*	**mean 0.6** *std. dev. 1.0*	p > 0.05	p > 0.05
SEBORRHOEA	**mean 1.1** *std. dev. 0.9*	**mean 1.0** *std. dev. 0.6*	**mean 0.9** *std. dev. 0.5*	p > 0.05	p > 0.05
ERYTHEMA	**mean 0.2** *std. dev. 0.4*	**mean 0.1** *std. dev. 0.3*	**mean 0** *std. dev. 0*	p > 0.05	p > 0.05
ITCHING	**mean 0.2** *std. dev. 0.6*	**mean 0.4** *std. dev. 0.8*	**mean 0.4** *std. dev. 0.8*	p > 0.05	p > 0.05
BURNING	**mean 0** *std. dev. 0*	**mean 0** *std. dev. 0*	**mean 0** *std. dev. 0*	---	---
TOLERABILITY	**- - -**	**mean 2.8** *std. dev. 0.6*	**mean 2.8** *std. dev. 0.6*	---	---

No statistically significant variation in the levels of dandruff, seborrhoea, erythema, itchng and burning sensation was detected during the period of treatment.

2 subjects referred about itching sensation after the product application; whatever the product was well tolerated.

The following table shows the comparison between the two products as regards the dermatological parameters.

AT09073 A vs AT09073 B	T_{45days} - T_0	$T_{3months}$ - T_0
DANDRUFF	p > 0.05	p > 0.05
SEBORRHOEA	p > 0.05	p > 0.05
ERYTHEMA	p > 0.05	p > 0.05
ITCHING	p > 0.05	p > 0.05
BURNING	p > 0.05	p > 0.05

No significant difference was detected in all considered parameters at both checks.

6.c. SUBJECTIVE EVALUATION

The results concerning the volunteers' self-assessment after the use of the products are reported below:

How do you judge the treatment as regards its efficacy in decreasing the hair loss?

	Number of answers (%) after 45 days		Number of answers (%) after 3 months	
	AT09073 A	AT09073 B	AT09073 A	AT09073 B
Very effective	3 (25.0%)	0 (0%)	4 (33.3%)	3 (25.0%)
Fairly effective	8 (66.7%)	8 (66.7%)	6 (50.0%)	4 (33.3%)
Not very effective	1 (8.3%)	4 (33.3%)	2 (16.7%)	5 (41.7%)
Not effective, at all	0 (0%)	0 (0%)	0 (0%)	0 (0%)

How do you judge the treatment as regards its pleasantness?

	Number of answers (%) after 45 days		Number of answers (%) after 3 months	
	AT09073 A	AT09073 B	AT09073 A	AT09073 B
Very pleasant	7 (58.4%)	1 (8.3%)	8 (66.7%)	3 (25.0%)
Fairly pleasant	4 (33.3%)	6 (50.0%)	3 (25.0%)	4 (33.3%)
Not very pleasant	0 (0%)	3 (25.0%)	0 (0%)	4 (33.3%)
Not pleasant, at all	1 (8.3%)	2 (16.7%)	1 (8.3%)	1 (8.3%)*

7. CONCLUSIONS

In order to evaluate the anti-hair loss efficacy of the products **AT09073 A, Ref.ISPE: 70/10/01 – 766/09** and **AT09073 B, Ref. ISPE 70/10/02 – 767/09**, 2 groups of 12 volunteers each applied the products on the scalp for 3 months.

The results of the instrumental and clinical evaluations showed the following results:

ANALYSIS OF THE PHOTOTRICHOGRAM

No statistically significant variation was recorded in the considered parameter for both products.

The phototrichogram analysis did not evidence any statistically significant difference between the two products.

OBJECTIVE DERMATOLOGICAL EVALUATION

AT09073 A: a statistically significant decrease in the level of seborrhoea was recorded at both control checks.

Both products were well tolerated.

SUBJECTIVE EVALUATION

AT09073 A: **91.7%** of the volunteers judged the treatment effective and pleasant after 45 days of treatment; at the end of the test **83.3%** judged the product effective and **91.7%** pleasant.

AT09073 B: **66.7%** of the volunteers judged the product effective and **58.3%** of the volunteers thought that the product was pleasant after 45 days of treatment; **58.3%** of the subjects judged the treatment effective and pleasant at the end of the test.

Responsible for the laboratory
Dr. Adriana Bonfigli

Responsible for the evaluation
Dr. Ludovica Mulone

Dermatologist
Dr. Fernanda Distante

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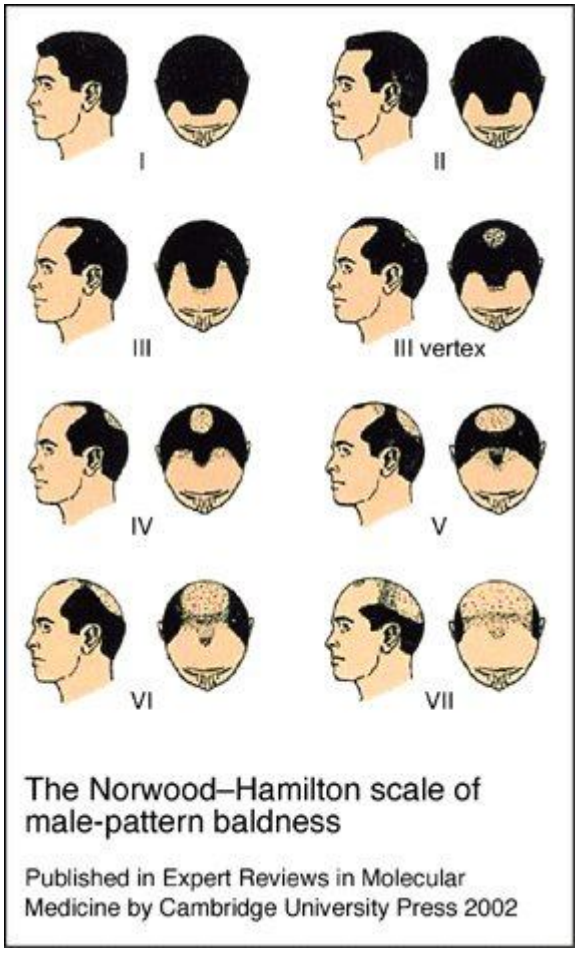
APPENDIX

BALDNESS SCALE

MALE PATTERN: NORWOOD-HAMILTON SCALE



The Norwood–Hamilton scale of male-pattern baldness

Published in Expert Reviews in Molecular Medicine by Cambridge University Press 2002

FEMALE PATTERN: LUDWIG SCALE


Grade I
Grade II
Grade III
Ludwig Hair Loss Scale