Bio Basic Europe S.r.l.
Uffici: Via Antonio Panizzi, 10 - 20146 Milano
Phone +39 02 4155729 - Fax 02 4150184
info@biobasiceurope.it - www.biobasiceurope.it
Sede Legale: C.so Monforte, 19 - 20122 Milano

ISCR. REG. SOC. TRIB. MI 1552357
CCIAA MI 1511487 – CAP. SOC. € 10.845.59
C.F- P.IVA 11930080152

SPF (PRELIMINARY TEST) DETERMINATION OF THE SOLAR PROTECTION FACTOR (SPF) ON A SMALL NUMBER OF VOLUNTEERS FOLLOWING COLIPA METHOD

Record no. 1303M26SP3

BIOSELECT

BIOSELECT
SUN EXPERT FACE CREAM FOR ALL SKIN TYPES
SPF 10

Place and date of issue: 10th December 2013

Bio Basic Europe S.r.l.
Uffici: Via Antonio Panizzi, 10 - 20146 Milano
Phone +39 02 4155729 - Fax 02 4150184
info@biobasiceurope.it - www.biobasiceurope.it
Sede Legale: C.so Monforte, 19 - 20122 Milano

ISCR. REG. SOC. TRIB. MI 1558357
CCIAA MI 1511487 – CAP. SOC. € 10.845,59
C.F- P.IVA 11930080152

EXPERIMENTAL PART

Title

Determination of the solar protection factor (SPF) of a cosmetic product on a small number of volunteers (no. 5 subjects) - COLIPA method.

Aim

To evaluate the protective effectiveness of a solar product towards damage caused by UV radiations.

Sponsor

BIOSELECT

Monitor

Prof. Plinio RICHELMI
Professor of Pharmacology
Director of the Medical Hydrology School
Director of the Pharmacology and Cellular and Molecular Toxicology Division
Department of Internal Medicine and Medical Therapy
Faculty of Medicine and Surgery
University of Pavia
Via Ferrata 9 - 27100 PAVIA
Italy
Tel. +39(0382)986346
Fax.+39 (0382)28426

Quality Control

Dr. Claudio ANGELINETTA
Technical Director at BIO BASIC EUROPE S.r.l.
Via A. Panizzi, 10 - 20146 MILANO
Tel. (02) 4155729
Fax (02) 4150184

Experimenter

Dr. Fernando Marco BIANCHI
Dermatologist
Consultant at CDC
Via A. Panizzi, 10 - 20146 MILANO

Report written by

Dr. Eliana Regola
Degree in Biological Sciences- Address of Molecular Biology and Development - University of Genoa
PhD in Experimental, Molecular and Clinical Biology and Medicine –Biochemistry Address- University of Genoa
Specialist in Microbiology and Virology-Technical address - University of Genoa
BIO BASIC EUROPE S.r.l.
Via A. Panizzi, 10 - 20146 MILANO
Tel. (02) 4155729 - Fax (02) 4150184

Bio Basic Europe S.r.l.
Uffici: Via Antonio Panizzi, 10 - 20146 Milano
Phone +39 02 4155729 - Fax 02 4150184
info@biobasiceurope.it - www.biobasiceurope.it
Sede Legale: C.so Monforte, 19 - 20122 Milano

ISCR. REG. SOC. TRIB. MI 1552357
CCIAA MI 1511487 – CAP. SOC. € 10.845,59
C.F- P.IVA 11930080152

DESCRIPTION OF THE METHOD FOR THE DETERMINATION OF THE SOLAR PROTECTION FACTOR (SPF)
(COLIPA SUN PROTECTION FACTOR TEST METHOD, 2006)

Introduction

The solar protection degree has traditionally been reckoned through the test of the solar protection factor (SPF), which evaluates the erythematous reaction of the skin to the ultraviolet radiation.
The solar protection factor (SPF) is the ratio between the necessary energies to produce the slightest erythematous reaction - with or without the application of a solar product on the volunteers' skin - using a ultraviolet radiation coming in most cases from an artificial source.
The COLIPA working team has examined all the aspects of the SPF test and has considered the possible causes of variation in the testing. The result was a method which can easily be carried out in the same way in all labs where the test is done.

Description of the method

The COLIPA method for the determination of the SPF is a lab method which uses an artificial source of ultraviolet radiation with a precise and definite output.
An increasing sequence of late erythematous reactions to the UV is produced on some small skin areas of the volunteers.
The volunteers undergo at least two medical examinations in the lab; first to expose themselves to the sequence of UV radiations and then for the evaluation of the late erythematous reaction.
Increasing incrementally the UV dose, different degrees of cutaneous erythema (redness due to surface vasodilation) are produced, reaching its maximum about 24 hours after UV exposure. A typical exposure time to produce erythema on an unprotected skin is 2 minutes
The minumum dose which produces an evident erythema is called "minimum erythematous dose" or MED. MED on unprotected skin (MEDu) and MED on protected skin (MEDp) are evaluated at the same time on the same volunteer.
MEDu and MEDp can be evaluated at sight by experts or instrumentally using a colorimeter.
More than a product can be tested on the same volunteer at any time.
The Solar Protection Factor (SPF) of the product is calculated for each volunteer using the ratio MEDp/MEDu.

Volunteers taking part in the test

The volunteers taking part in the test are selected from a panel of subjects including skin phototypes I, II and III.
They undergo a medical inspection in which the doctor examines their state of health and verifies their suitability to take part in the test.
All volunteers have to sign a written consent, if they decide to partecipate.
The exclusion criteria are the following:
- pregnant and nursing women
- subjects under treatment (for instance: under pharmacological treatment)
- subjects suffering from dermatopathies
- subjects who had abnormal reactions to sun exposure
- subjects who made a long use of UVA sun beds

Bio Basic Europe S.r.l.
Uffici: Via Antonio Panizzi, 10 - 20146 Milano
Phone +39 02 4155729 - Fax 02 4150184
info@biobasiceurope.it - www.biobasiceurope.it
Sede Legale: C.so Monforte, 19 - 20122 Milano
ISCR. REG. SOC. TRIB. MI 1558357
CCIAA MI 1511487 – CAP. SOC. € 10.845,59
C.F- P.IVA 11930080152

UV radiation source

There's a UV radiation source, a filter system to change the spectrum output, and devices to measure exposure flux and exposure time, so that the spectrum is continuous, uninterrupted and there aren't any extreme peaks of radiation in the UV area.
To ensure that appropriate amounts of UVA radiation are included in the spectrum of the solar simulator throughout the entire UVA range, the total radiometric proportion of the UVA II (320-340 nm) irradiance of the simulator must equal or exceed 20% of the total UV (290-400 nm) irradiance. Additionally, the UVA I region (340-400 nm) irradiance must equal or exceed 60% of the total UV irradiance.

Products with standard SPFs

The standard product, which is generally used, is the following:
• P3: Mean value SPF= 16.2 (Range 13.8-18.7).

Application area of the product
The smallest area, on which product has been applied, was 50 cm^2.
The unprotected area for the determination of MEDu is near to the areas for the determination of MEDp.
The areas for the determination of MEDp are on the back. This for all volunteers to avoid variations concerning skin anatomy differences. Contiguous application areas are at least 1 cm. apart.
The application area/areas should be outlined with a form in non-absorbent material.

Product quantity applied
The standard and the tested products have been applied in a 2.00$\pm$ 2.5% mg/cm^2 quantity.
Leaks due to evaporation of volatile components after weighting of the product and before its application have been avoided.

Product application
Small quantities of the product are applied on the whole test area, using a suitable instrument. The area is softly massaged with a smooth glove to obtain a uniform application.
There shouldn't be any accumulation of the product at the form extremes (if a form has been used). A clean glove is to be used for each product.
The skin is sometimes moistened, especially when powders are used, to allow application of the whole standard product dose.

Waiting time between product application and UV exposure

The tested areas have been exposed to UV radiations 15 minutes after application of the product

Subjects'position

During UV exposure the subjects were sitting down. They were instructed not to move their arms or change position during this period.
The position was the same both for the UV exposure and for the MED evaluation.

Incremental progression of the UV dose

The exposure areas were at least five. The UV doses were incremental (geometric progression of 1.12).

Bio Basic Europe S.r.l.
Uffici: Via Antonio Panizzi, 10 - 20146 Milano
Phone +39 02 4155729 - Fax 02 4150184
info@biobasiceurope.it - www.biobasiceurope.it
Sede Legale: C.so Monforte, 19 - 20122 Milano

ISCR. REG. SOC. TRIB. MI 1558357
CCIAA MI 1511487 – CAP. SOC. € 10.845,59
C.F- P.IVA 11930080152

MED evaluation

MED was evaluated 20±4 hours after exposure. (This is the best time to evaluate possible erythematous reactions). The minimum erythematous dose on unprotected skin (MEDu) and MED on protected skin (MEDp) were evaluated simultaneously.
The visual evaluation was made when lighting was uniform and good.
A first MED evaluation was made on a small sample of volunteers (no. 5 volunteers). After that MEDu and MEDp were simultaneously evaluated.

Bio Basic Europe S.r.l.
Uffici: Via Antonio Panizzi, 10 - 20146 Milano
Phone +39 02 4155729 - Fax 02 4150184
info@biobasiceurope.it - www.biobasiceurope.it
Sede Legale: C.so Monforte, 19 - 20122 Milano
ISCR. REG. SOC. TRIB. MI 1558357
CCIAA MI 1511487 – CAP. SOC. € 10.845,59
C.F- P.IVA 11930080152

MED expression

MED is expressed as time (seconds).

Reckoning of the Solar Protection Factor (SPF)

When the 95% CL was met, the SPF of the product was expressed as the average of the SPFi values of all volunteers who took part in the test.
The general equations are the following:

Individual Solar Protection Factor (SPFi)

SPFi = MEDpi / MEDui

Solar Protection Factor of the Product (SPF)

SPF = (□SPFi) / n

Its standard deviation (s) is:

$$s = \sqrt{\frac{\sum (SPFi)^2 - \frac{\left(\sum SPFi\right)^2}{n}}{n-1}}$$

95% Interval confidence (CL)

95% CL = from SPF - c to SPF + c

$$c = \frac{ts}{\sqrt{n}}$$

where t = t value taken from the bilateral chart concerning the Student distribution with a probability level p = 0.05 and with degrees of freedom □= (n-1)

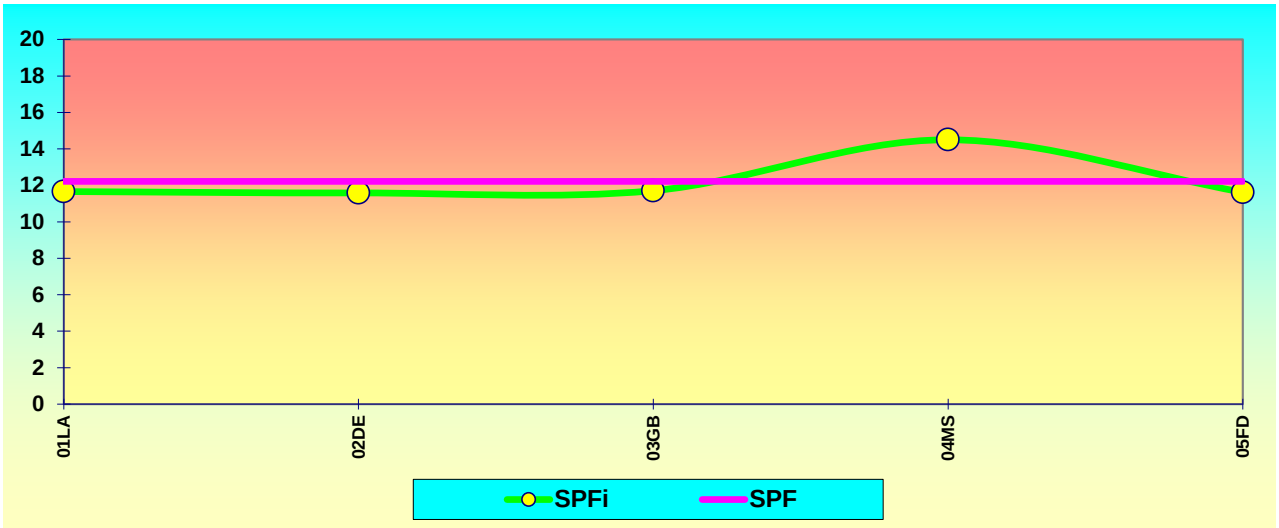
N	5
t	2,776

Bio Basic Europe S.r.l.
Uffici: Via Antonio Panizzi, 10 - 20146 Milano
Phone +39 02 4155729 - Fax 02 4150184
info@biobasiceurope.it - www.biobasiceurope.it
Sede Legale: C.so Monforte, 19 - 20122 Milano
ISCR. REG. SOC. TRIB. MI 1552357
CCIAA MI 1511487 – CAP. SOC. € 10.845.59
C.F- P.IVA 11930080152

Report no. **1303M26SP3** **BIOSELECT**

SUMMARIZING TABLES

reference	SEX	Phototype	MEDu sec.	MEDp sec.	SPFi	SPF	s	c	CL (0,17 SPF)
01LA	F	I	35	408	11,7				
02DE	M	II	55	637	11,6				
03GB	M	II	85	995	11,7				
04MS	F	III	134	1943	14,5				
05FD	F	III	209	2429	11,6	**12,2**	**1,3**	**1,3**	**2,1**
Final result with variability							10,9	**12,2**	13,5



Bio Basic Europe S.r.l.
Uffici: Via Antonio Panizzi, 10 - 20146 Milano
Phone +39 02 4155729 - Fax 02 4150184
info@biobasiceurope.it - www.biobasiceurope.it
Sede Legale: C.so Monforte, 19 - 20122 Milano

ISCR. REG. SOC. TRIB. MI 1552357
CCIAA MI 1511487 – CAP. SOC. € 10.845.59
C.F- P.IVA 11930080152

CONCLUSIONS

From the tables and the charts listed above, we can maintain that the product tested on a small number of volunteers - no. 5 subjects - following the COLIPA method for the determination of the Solar Protection Factor has got the following SPF:

BIOSELECT
SUN EXPERT FACE CREAM FOR ALL SKIN TYPES
SPF 10

12.2 ± 1.3

BIO BASIC EUROPE
Quality Control

Dr. Claudio Angelinetta

Bio Basic Europe S.r.l.
Uffici: Via Antonio Panizzi, 10 - 20146 Milano
Phone +39 02 4155729 - Fax 02 4150184
info@biobasiceurope.it - www.biobasiceurope.it
Sede Legale: C.so Monforte, 19 - 20122 Milano
ISCR. REG. SOC. TRIB. MI 1558357
CCIAA MI 1511487 – CAP. SOC. € 10.845.59
C.F- P.IVA 11930080152

Report no. **1303M26SP3** **BIOSELECT**

STANDARD P3

reference	SEX	Phototype	MEDu sec.	MEDp sec.	SPFi	SPF	s	c	CL (0,17 SPF)
01LA	F	I	35	509	14,5				
02DE	M	II	55	796	14,5				
03GB	M	II	85	1554	18,3				
04MS	F	III	134	1943	14,5				
05FD	F	III	209	3795	18,2	**16,0**	**2,0**	**2,1**	**2,7**
Final result with variability							13,9	**16,0**	18,1