A Double-Blind, Randomized Placebo-Controlled Study to Access the Safety and Efficacy of an Over-The-Counter, At-Home Lip Enhancer Device Intended to Induce Lip Plumping

Sponsor: CandyLipz LLC.
Protocol: CAZ050217DVCA
Clinical Phase: IV
Name of Test Device: CandyLipz Lip Plumping System
Third-Party Clinical Trial Investigative Firm: Biometrix Inc. San Francisco, California U.S.A.

INTRODUCTION

Plump, full lips are the hallmarks of youth and beauty. Increasingly, women are turning to plastic surgery and other physician office procedures, such as filler injections (Funt and Pavicic, 2013). There are negative aspects to these procedures, including potential adverse events such as tissue necrosis (Dayan et al., 2011; Grunebaum et al., 2009; Kwon et al., 2013), granuloma formation (Lemperle et al., 2008; Alijotas-Reig et al., 2008; Chrastil-LaTowsky et al., 2009) and inflammatory nodules (Sclafani et al., 2009). For most, the cost of the procedure and the need to follow up at a doctor's office are major deterrents to those who want fuller lips.

This study evaluated an inexpensive, at-home treatment to enhance the fullness and plump appearance of lips – the CandyLipz Lip Plumping System (Thienna 2011). Its mechanism is based on "cupping," which is a 3,500-year old healing method that dates back to ancient China. This treatment method was historically considered a very beneficial procedure and in the 1950s, Chinese hospitals used cupping to treat many conditions. Research has demonstrated favorable results for cupping therapy. It treats conditions such as herpes zoster, facial paralysis (Bell's Palsy), cough and dyspnea, lumbar disc herniation, pain and cervical spondylosis (Cao et al. 2012, Kiefer 2016). It also improves circulation, treats acne and other skin conditions and disorders (Kiefer 2016). In 2016, cupping marks made major headlines in the news from the 2016 Rio Olympic Games. Olympic gold medalist, Michael Phelps, and other U.S. team athletes practiced cupping to help circulate blood and relieve muscle tension (Josh 2016).

The most common traditional 'cupping' method uses glass cups with heat to create suction on local skin.

Instead of using glass cups, CandyLipz lip plumping method uses hand-operated suction silicone tubes/bulbs with an internal mouth piece to shape, contour, and plump lips naturally and without needles.

ABSTRACT

This cupping therapy or treatment can produce "cupping marks" on treated local areas. Cupping mark colors can range from bright red to dark purple**.** Some CandyLipz Plumping System users have reported such marks. This clinical study sought to determine the effects of cupping in lip and lip area tissue as well as to define the physiological state of skin afflicted with cupping marks.

Over a 56-day period, the study evaluated approximately 50 women who were assigned use of the test device, in accordance with typical use instructions, and 25 women using the test device without suction (placebo control). As a control, the same device was used without the application of pressure. In order to closely examine the biophysical, physiological, hematological and histological effects of lip cupping, this clinical trial evaluated treated skin by multiple clinical methodologies (see definitions at end below).

We measured the appearance of dermal vasculature by image analysis, evidence of objective and subjective irritation, color of the lips and the area above the upper lip, blood perfusion, skin barrier properties and structural aspects of the treated lips and lip area skin by ultrasound and by histological analysis of treated skin tissue samples.

Each of the following methods demonstrated the system to be safe:

- Dermatologist visual grading
- Collection of subjective responses
- TEWL measurements
- Ultrasound imaging and blue light image analysis of the treated lip area
- Histological analysis of tissue samples from forearm skin treated with CandyLipz Lip Plumping System under exaggerated use conditions.

The device did not elicit any bruising, capillary breakage or red blood cell leakage in the treated skin. The dermatologist concluded that any lip cupping marks, which were more prominent in subjects with more noticeably increased plumping, were transient in nature and for most, resolved within 24 hours. Skin barrier health, as measured by transepidermal water loss (TEWL), was improved with use of the device. Ultrasound images did not exhibit evidence of any structural changes to the skin, which was consistent with histological analysis. The pathologist evaluating the tissue samples of skin treated under exaggerated conditions stated that any lip cupping marks were attributed to the resurgence of blood to the skin area following displacement by the pressure elicited by the device. He compared this to pressing a finger against the skin. The skin showed a temporary "white mark" where the finger contacted the skin, and the blood subsequently returned. The influx of blood would appear as erythema following the negative pressure induced by the lip cupping system. This result is substantiated by Laser Doppler measurement of blood flow.

The study also aimed to determine if the test device was an effective option for improving the fullness and plumpness of the lips. This efficacy was not substantiated by subject self-assessment questionnaire response proportion analysis. However, image analysis with a 3-D imaging system (Primos$^{Lite™}$) demonstrated significant decreases in lip lines, an inverse measure of lip plumping as well as improved lip contrast. This result indicates that use of the CandyLipz Lip Plumping System plumped and smoothed the lips significantly. Dermatologist assessment of changes in lip contrast, smoothness and fullness demonstrated statistically significant increases in each of these attributes in the treated group.

RESULTS AND DISCUSSION

It's been proven that under this study's conditions, when used as directed, the CandyLipz Lip Plumping System is safe. Safety was assessed over a 56-day treatment period in accordance with use instructions outlined in Appendix 1. Evaluations of safety included:

- Blue light analysis of VISIA images
- Dermatologist grading of objective irritation
- Collection of subjective reports of irritation
- Measures of color with the chromameter and spectrophotometer
- Blood flow by Laser Doppler
- Transepidermal water loss and ultrasound imaging

From the data collected in this study, it's evident that there's no damage to skin tissue as a result of lip cupping. While some subjects did exhibit "cupping marks" in the form of an erythemal response, it was determined that these marks are temporary and were observed in the absence of any pain or other discomfort reported by subjects. These marks were determined to result from no structural skin change or bruising (hemorrhaging).

Blue light analysis of images demonstrated that there was no significant increase in blue color, which would have been the result had there been bruising, hemorrhaging or venous breakage of any type. The cupping marks are the result of a temporary displacement of blood during use, as would be the case for application of any pressure to the skin, and the consequential resurgence of blood to the treated area. Not only was the device determined to elicit no consequential indications of irritation, but there was also evidence of an improvement in skin barrier.

Increases in dermatologist-assessed symptoms of irritation were minimal, less than one score level on average, and did not persist beyond Day 9 of the study. These symptoms included erythema and edema, which were expected to increase from use of the test device as a normal side effect of all lip plumping methods (Funt and Pavicic 2013). The study dermatologist reported that any lip cupping marks observed were in subjects exhibiting greater plumping effects than other subjects and that these were mild and transient.

Spectrophotometer readings of the skin area above the lip indicated increases in redness at the treated sites, with increases at time points consistent with those of the objective irritation observations. Chromameter readings of the lips did not exhibit any significant color change. There were minimal, however, statistically significant, increases in the appearance of dryness of the lips and lip area. This may be attributable to increased lip volume eliciting a cracked appearance in the most superficial layer of skin, which is chronically dry under normal situations (Freeman 2013).

The study pathologist, who examined excised tissue samples of forearm skin treated under exaggerated use conditions relative to untreated excised skin, hypothesized that the transient increases in erythema or other visual appearance consistent with "cupping marks" was due to vascular effects of the pressure imposed by the device. This is consistent with Laser Doppler blood flow results, which showed significantly decreased rates of red blood cell movement at treated lips immediately (30 minutes) after plumping. The influx of blood to the treated area appears visually as erythema. With the extended period of pressure elicited by the device, it is not unusual that the blood flow, appearing as erythema, returns to normal at the treatment site 24 hours after treatment. This was substantiated by the Laser Doppler results at 24 hours. Similarly, at Day 9, evaluations assessed this same effect at approximately 12 to 18 hours after test device use.

Subjects did not perceive any significant increase in symptoms of subjective irritation, which included stinging, burning, itching, tingling and pain, following use of the device and throughout the study period. It should be noted that even in subjects with erythema and edema increases relative to baseline, there was no reported pain or any other perceived indication of irritation or discomfort.

The skin barrier on the lip not only remained intact, with no increases in transepidermal water loss (TEWL), but exhibited improvement. There were statistically significant decreases in all post-treatment TEWL values in the group using the device in accordance with the instructions with suction applied. This implies that the skin barrier improved during use of the test article. It was anticipated that TEWL would decrease due to application of the Vaseline Petroleum Jelly in between CandyLipz Lip Plumping System use. However, these improvements were not measured for the placebo group, who also applied Vaseline Petroleum Jelly in the same manner, suggesting that the suction application use of CandyLipz Lip Plumping System improved skin barrier function.

Ultrasound images collected above the upper lip did not exhibit any perceptible skin structural changes in the treated or placebo group at post-treatment intervals relative to baseline. Furthermore, histological analysis of forearm skin samples treated under exaggerated use conditions did not show any difference from untreated skin. There was no evidence of tissue damage of the epidermis or dermis. Additionally, there was no sign of vascular hemorrhaging, as is seen in the case of bruised tissue, nor any capillary breakage or leaking of red blood cells.

Efficacy of CandyLipz Lip Plumping System was assessed by questionnaire response proportion analysis; color contrast assessment from VISIA images; dermatologist grading of lip contrast, smoothness and fullness; and three-dimensional inverse analysis of plumping from Primos^{lite} High Resolution Small Field Capture Imaging.

The efficacy of CandyLipz Lip Plumping System to plump and improve the lips was substantiated by image analysis with a 3-D imaging system (Primos$^{Lite™}$). Image analysis measurements of lip lines decreased significantly. Lip line measurements are an inverse measure of lip plumping, demonstrating a significant increase in plumping, as well as an increase in lip smoothing. Additionally, color contrast image analysis of the lip and surrounding skin increased significantly in the treated group using the Lip Plumping System in accordance with the instructions. Subject self-assessment questionnaire response proportion analysis showed that the majority of subjects perceived improvements from device use.

Dermatologist assessment of changes in lip contrast, smoothness and fullness demonstrated statistically significant increases in each of these attributes in the treated group.

CUPPING MARKS INTENSITY

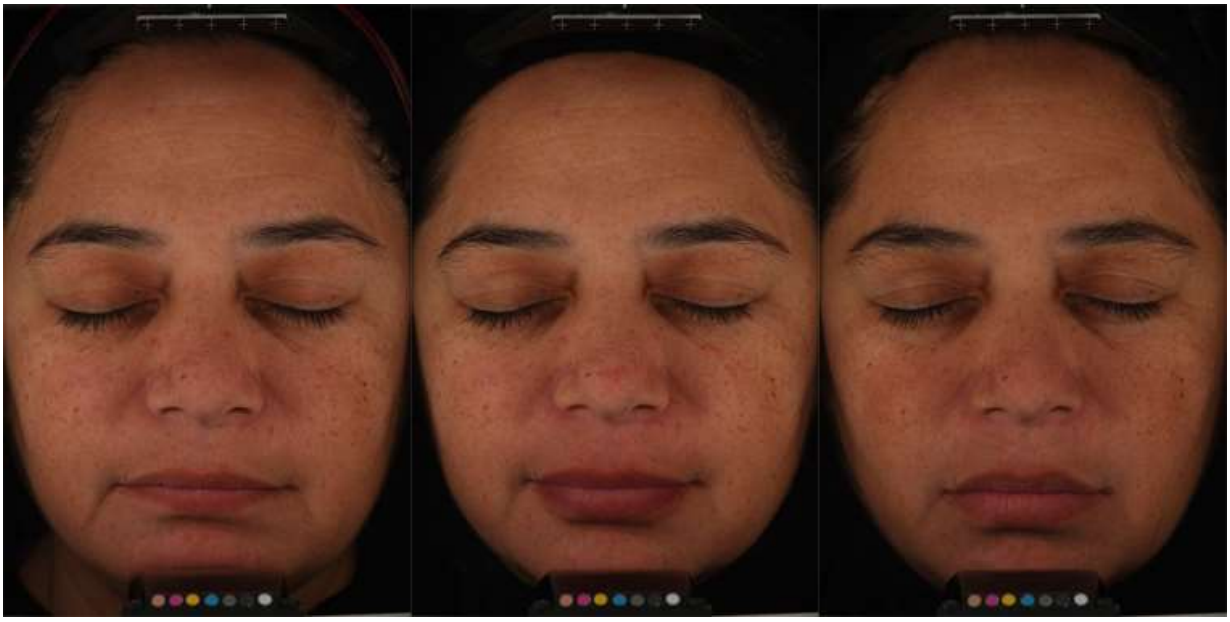
The image above shows cupping marks, 24 hours after device use. Whether mild, moderate or severe, these marks were transient and were not seen by Day 28 and through Day 56. In the treated group, the results showed 31% had no marks (1st image); 45% had mild marks (2nd image); 19 % had moderate marks (3rd image); and 5% had intense marks (4th image).

95% of subjects showed cupping marks resolved between Day 1 and Day 9. By Day 56, lips appeared smooth and plumper.

RESULTS AFTER 24 HOURS AND 56 DAYS

The image set below showed the CandyLipz Lip Plumping System validated results of natural lip enhancement from water accumulation and retention. Image 1 (left) showed

baseline lips without plumping. Image 2 (center) showed lips were still plump after 24 hours without plumping. Image 3 (right) showed lips are fuller naturally without plumping at Day 56.



SUMMARY OF THE STATISTICALLY VALIDATED FINDINGS

CandyLipz Lip Plumping - Claims

CLAIMS	Statistically validated	Times validated
No bruising, broken vessels, vascular hemorrhage or broken red blood cells	YES	-
Increase of lip visibility	YES	24h/9/28 days
Increase of blood flow	YES	Immediate
Increase of lip tissue vascularization (formation of blood vessels)	YES	Imm./24h/56 days
Increase of lip volume	YES	Imm./24h/9 days
Decrease of lip lines and wrinkles (increase in tension and smoothing)	YES	Imm./24h/9 days
Increase of volume by water accumulation and retention	YES	Imm./24h

The table above showed CandyLipz validated claims. A total of 11 methodologies were carried out in the clinical study of cupping marks (Appendix 2): Blue Color Image Analysis, RGB Digital Imaging Analysis, Dermatologist Grading , Chromameter Readings, Laser Doppler Readings, Tewameter® TM300 (C+K, Germany) Measurements, Spectrophotometer measurements, Ultrasound Imaging, Primoslite High Resolution Small Field Capture Imaging, VISIA Contrast Image Analysis, and Subject questionnaire responses and Subject Satisfaction Scale.

Appendix 1:
Use Instructions

First Use at the Test Center – Exaggerated

- Put the device to your lips and align the opening of the device with the center of your lips. If you are using a creaser mouth opening, you need to make sure that the creaser is centered at the midline of your lower lip.
- Next, pucker your lips as far out as possible and hold them together. Learn not to drip saliva into your device. Do not breathe into the product or let air enter it. Breathe through your nose to avoid the problem.
- Leave the lip device in place for 1 minute.
- After 1 minute, remove the device from your lips. Rest for 2 minutes. During the rest period, massage the lips gently for 1 or 2 minutes with Vaseline. Dab off the Vaseline before you repeat use.
- You will put the device on your lips for a total of 5 more rounds for 1 minute each round with a rest period of 2 minutes in between.

Day 1 to Day 8 (Conditioning Period):

- Rest your lips on Days 1 to 2.
- On Days 3 to 5, use the plumper once daily for one minute with a two-minute break and then repeat one more use of the plumper.
- On Days 6 to 8, use the plumper once time daily. For each use, put the lip plumper to your mouth for two minutes, take a ten-minute break and then repeat two more uses of the plumper with a 10-minute rest in between.
- During the rest periods, apply Vaseline.

Remainder of Study:

- Use the plumper once daily. For each use, plump for 1 minute, rest for two minutes; apply Vaseline during rest period, then plump for another minute.

Appendix 2:
STUDY METHODOLOGIES

Blue Color Image Analysis

VISIA images captured in cross-polarized light allow for the visualization of the vasculature underlying the skin. The detection of blue light in cross polarized images that are analyzed following capture can indicate the presence of broken capillaries. Red coloration detection indicates that the capillaries are intact.

RGB Digital Imaging Analysis

Digital Image Analysis software applied to VISIA images captured at Baseline and 24 hours. The area of skin evaluated was the skin surrounding the lip area of each subject. Since previous studies on lip cupping have identified the potential for "hickey marks" to occur, the measurements utilized the skin in the area on which "hickey marks" are likely to occur. This is the area above the upper lip, but it is sometimes below the lower lip. The images from baseline were compared side by side with the post-treatment intervals.

The software measures color in an RGB Color Space. It is a three-dimensional space and the values for R, G and B (red, green and blue) are the coordinates for the location of the measured color in that color space. This color method allows for detection of bruising in the images.

Dermatologist Grading

The Dermatologist/Investigator examined the lips of each subject to ensure the absence of erythema, edema and dryness or any other observed symptom of irritation. A medical office lamp was used to illuminate the skin of each subject with consistent evenly diffused lighting.

Dermal scores for the lip area were recorded on a source document (see scoring scales listed at the bottom of the dermal scoring data tables). Forearm skin was examined to ensure eligibility for skin biopsy candidates. At the baseline examination, subjects exhibiting visual symptoms of irritation, at any level, were excluded from participation.

Chromameter Readings

The Chromameter is a handheld instrument that has a computer interface to collect and store color measurements. The instrument has a round, plastic (Plexiglas) disc that is placed against the skin. For this study, the instrument was placed on both the upper and lower lips (held together with a closed mouth). A pulse of light of a fixed illumination is sent to the skin surface, and the Chromameter instantly reads the color that is returned, reflected from the skin surface.

Colorimetric values are collected by the Chromameter in a three-dimensional color space for which the values expressed by the instrument, L*, a* and b*, relate to numerical color coordinates. L* is the component that relates to grey scales ranging from theoretical blackest color (value 0) to whitest color (value 100). The a* value relates to opposing spectrum of red (positive numbers) and green (negative numbers); and b* measures the color value from yellow orange (positive numbers) to blue (negative numbers). Increased a* values would indicate increased lip redness, which may be an indication of erythema.

Laser Doppler Readings

The Laser Doppler measures the rate of blood flow in terms of number of red blood cells passing through the measurement area in a unit of time. Increased perfusion measurements have been associated with surface pressure increases and elevated blood vessel density.

Tewameter® TM300 (C+K, Germany) Measurements

The Tewameter® TM300 (C+K, Germany) is a device that measures transepidermal water loss (TEWL). It is a measure of the skin barrier integrity. The measurement is related to the water content in the microenvironment directly above the skin surface. It increases when more water is transpired from the skin. When the top layers of skin become damaged, for example, by use of a strong surfactant (cleanser) or abrasion, the water lost transepidermally increases (higher TEWL).

In this study, TEWL readings were used to assess damage to the skin of the lips after treatment with the evaluated devices. One measurement was taken from the lower lip.

Spectrophotometer measurements

The Spectrophotometer is a handheld instrument that has a computer interface to collect and store color measurements. The instrument has a round, plastic (Plexiglas) disc that is placed against the skin. For this study, the instrument will be placed above the upper lip. This is the location that previous studies on lip cupping exhibited “hickey

marks" in some subjects tested. A pulse of light of a fixed illumination is sent to the skin surface, and the instrument instantly reads the color that is returned, reflected from the skin surface.

Ultrasound Imaging

High resolution ultrasound (25MHz) can be used on lips to determine the density of the connective tissue in the skin. Potential vascular extravasation of blood in tissues is detectable by this method.

Primos^{lite} High Resolution Small Field Capture Imaging

The Primos imaging system allows for the captures a small field image. The image analysis software that is installed in the system measures small microstructural features of the skin. In this study, images of the lips were captured at focal points that allowed for demonstration of lip surface roughness. Volumetric measurements cannot be determined by this imaging system. However, changes in roughness are inversely proportional to volumetric changes.

VISIA Contrast Image Analysis

VISIA images collected from study time points were analyzed after study conclusion. Lip contrast was quantified by image analysis assessing the color darkness difference between the lips and the surrounding skin.

Subject questionnaire responses

In order to assess product perception for each of the devices tested, subjects were asked to respond to a questionnaire and complete at Subject Satisfaction Scale.

Skin Biopsy Samples

Skin biopsy samples were collected from a sub-population of subjects. At the baseline visit, two sites were identified on the forearm of participating subjects (n=5, plus control – aging skin). One of the two sites was treated with CandyLipz Lip Plumping System with exaggerated use procedures. A bandage treated with lidocaine was applied securely to the skin of each of the treated and untreated sites to anesthetize the skin for biopsy the following day.

Upon returning at 24 hours after device use, patches were removed from the forearm sites, and the skin was cleansed with alcohol. Sites were injected with local anesthesia,

and biopsy samples (3 millimeters in diameter) were taken from the treated and untreated sites. The samples were placed in a solution of 10% formalin and sealed in a glass jar. Samples were sent to Boston University's Pathology Laboratory for analysis to evaluate the effect of cupping on the skin at a structural level in terms of inflammation and/or tissue damage.

FDA COMPLIANCES

GCP Statement

This study was conducted in compliance with the study protocol, ICH E6: Good Clinical Practice: Consolidated Guidelines, Biometrix Inc. Standard Operating Procedures and all other applicable regulatory guidelines. The study protocol was reviewed and approved by Allendale Institutional Review Board. Biometrix Inc. was monitored by Biometrix Inc. Quality Assurance ensures that informed consent was obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a, b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA), and local regulations.

IRB Information

Protocol, consent form, advertising materials, test device information and investigator selection was reviewed and approved by the IRB listed below prior to study initiation:

Allendale Investigational Review Board
30 Neck Road
Old Lyme, Connecticut 06371
Robert J. Staab Ph.D., Chairman

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