

**JOHNSON & JOHNSON CONSUMER INC.**

## SUMMARY CLINICAL STUDY REPORT

PROTOCOL TITLE:	A Study of 3 Investigational Products on the Microbiome and Scalp Attributes in Children with Curly Hair
PROTOCOL NUMBER:	CCSSKB004136 Version 01 dated 05-Oct-2021
SITE STUDY NUMBER:	090666-01/03
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STUDY INITIATION DATE (First Subject First Visit):	13-Nov-2021
STUDY COMPLETION DATE (Last Subject Completed):	20-Jan-2022
SITE APPROVAL:	See trial master file for final clinical study report and approvals
SPONSOR REVIEW AND APPROVAL:	See trial master file for final clinical study report and approvals

The principles of the International Council for Harmonisation (ICH) Guidelines for Good Clinical Practice (GCP E6 (R2)) were applied to this study.

CONFIDENTIAL: The information in this document contains trade secrets and commercial information that are privileged or confidential and may not be disclosed unless such disclosure is required by Federal or State law or regulations. Subject to the foregoing, this information may be disclosed only to those persons involved in the study who have a need to know, but all such persons must be instructed not to further disseminate this information to others. These restrictions on disclosure will apply equally to all future information supplied to you, which is indicated as privileged or confidential.

<p><b>INTRODUCTION</b></p>	<p>The investigational products (IPs) are shampoo, conditioner, and combing cream (Routine kit).</p> <p>The human skin including the scalp surface, serves as the body's first line of defense as well as a host to a myriad of microorganisms, which includes both bacteria and fungi<sup>1</sup>.</p> <p>Global studies have revealed that the scalp microbiome is characterized by a rather low bacterial diversity, as compared to the other body sites, and the predominant microorganisms are <i>Cutibacterium acnes</i> (formerly <i>Propionibacterium acnes</i>), <i>Staphylococcus epidermidis</i> and <i>Malassezia</i> spp.<sup>1</sup>.</p> <p>The application of sequencing methods and robust computational analysis has led to an in-depth understanding of the scalp microbiome in the recent years, providing novel clues on the pathophysiology of scalp-related disorders. However, whether the scalp microbiome variation is a cause or a consequence of the unhealthy condition of the scalp remains unclear<sup>1</sup>.</p> <p>Due to the recently established role of the microbiome in skin and scalp health, some studies have investigated the effect of emollients and topical medications on the skin microbiome and the correlation of the microbiome with changes in host physiological parameters, such as TEWL (trans-epidermal water loss)<sup>1</sup>.</p> <p>Presenting a healthy scalp is a relevant attribute for consumers of cosmetic products, and this attribute must be supported by appropriate clinical studies.</p>
<p><b>OBJECTIVE</b></p>	<p>Primary objectives:</p> <p>Before and after 28 ± 2 days of using the cosmetic products kit (shampoo, conditioner, and combing cream), under normal use conditions, were evaluated:</p> <ul style="list-style-type: none"> <li>• Topical safety (tolerability/acceptability) supervised by a Dermatologist and a Pediatrician;</li> <li>• The presence of residues on the scalp by a trained technician using the residue counting method;</li> <li>• The scalp skin barrier through instrumental measurements with the equipment Tewameter® TM Nano (Courage + Khazaka Electronic GmbH);</li> <li>• The sebum level of the scalp through instrumental measurements with the equipment Sebumeter® SM 815 (Courage + Khazaka Electronic GmbH);</li> <li>• The scalp pH through instrumental measurements with the portable pH equipment (Seven 2 Go Mettler Toledo S8);</li> <li>• The capture of standardized images of the scalp using Fotofinder equipment to register the presence of possible residues.</li> </ul> <p>Secondary objectives:</p> <p>Before and after 28 ± 2 days of using the cosmetic products kit (shampoo, conditioner, and combing cream), under normal use conditions, were evaluated:</p> <ul style="list-style-type: none"> <li>• The Scalp Microbiome through non-invasive collection method and metagenomic analysis of fungi and bacteria;</li> <li>• The perception of parents/LAR (Legally Acceptable Representative) through a Quality-of-Life Questionnaire;</li> <li>• The scalp hydration through instrumental measurements with the equipment DermaLab® USB Moisture Module (Cortex Technology). This was an exploratory evaluation.<b>Error! No bookmark name given.</b></li> </ul>

<b>STUDY DESIGN</b>	The study protocol referenced on page 1 of this report provides the complete study design for the study.									
<b>SUBJECT INFORMATION</b>	<p>The complete eligibility criteria for this study were followed as defined in the study protocol referenced on page 1 of this report.</p> <p>The main criteria were:  Female and male children subjects, 3 to 6 years old, phototype (Fitzpatrick) IV to VI, curly to kinky hair (classification 3B to 4C) and with a minimum hair length of 5 cm (3 fingers).</p>									
<b>INVESTIGATIONAL STUDY MATERIALS</b>	<table border="1" data-bbox="678 495 1341 737"> <thead> <tr> <th data-bbox="678 495 1044 541">Identification</th> <th data-bbox="1044 495 1341 541">Product Type</th> </tr> </thead> <tbody> <tr> <td data-bbox="678 541 1044 604">Shampoo</td> <td data-bbox="1044 541 1341 604">Investigational Product (IP)</td> </tr> <tr> <td data-bbox="678 604 1044 667">Conditioner</td> <td data-bbox="1044 604 1341 667">Investigational Product (IP)</td> </tr> <tr> <td data-bbox="678 667 1044 737">Combing Cream</td> <td data-bbox="1044 667 1341 737">Investigational Product (IP)</td> </tr> </tbody> </table>		Identification	Product Type	Shampoo	Investigational Product (IP)	Conditioner	Investigational Product (IP)	Combing Cream	Investigational Product (IP)
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<b>DOSE AND MODE OF APPLICATION</b>	<p>The application of the IPs was performed or supervised by the subjects' parents/LAR at home.</p> <p>The product kit was used for 28 ± 2 days, at least 3 times a week, totaling at least 12 uses of the complete routine (Shampoo, Conditioner, and Combing Cream), according to the following instructions:  Give preference to short baths lasting between 5 and 10 minutes, if possible, using warm water. Use shower, not bathtub, for the baths.</p> <p><b>Shampoo:</b>  This should be the first investigational product to be used.  Instructions for Usage:</p> <ul style="list-style-type: none"> <li>• Apply shampoo to wet hair and scalp, massaging gently until lather is obtained;</li> <li>• Then rinse with water;</li> <li>• The amount to be applied will depend on the length and volume of your hair.</li> </ul> <p><b>Conditioner:</b>  This product should be used after the shampoo.  Instructions for Usage:</p> <ul style="list-style-type: none"> <li>• After using the shampoo, apply the conditioner to wet hair, lock by lock from the lengths to the ends, untangling the strands from bottom to top (from the ends to the root);</li> <li>• Leave for 1 to 4 minutes;</li> <li>• Then rinse with water;</li> <li>• The quantity to be applied will depend on the length and volume of your hair.</li> </ul> <p><b>Combing Cream:</b>  This investigational product should be used last, after shampooing and conditioning, and, if necessary, the next day(s) after washing your child's hair.  Instructions for Usage:</p> <ul style="list-style-type: none"> <li>• Apply the combing cream to wet or previously moistened hair;</li> <li>• Apply the cream lock by lock from the lengths to the ends.</li> <li>• Then scrunch the curls with upward and downward movements;</li> </ul>									

	<ul style="list-style-type: none"> <li>• Repeat this movement as many times as necessary;</li> <li>• Do not rinse;</li> <li>• The amount to be applied will depend on the length and volume of your hair;</li> <li>• Let the hair dry naturally or with the aid of a hair dryer;</li> </ul> <p>Do not use any hair care products other than those provided in this study during the period of 28 ± 2 days.</p>
<p><b>METHODOLOGY</b></p>	<p>The study contemplated the use of IPs for 28 ± 2 days at home, with 2 visits, totaling an estimated 4 weeks of study for each subject:</p> <p>Visit 1 – Day 1:</p> <ul style="list-style-type: none"> <li>• Screening and Enrollment</li> <li>• Initial Clinical Assessment</li> <li>• Scalp Microbiome Collection</li> <li>• Scalp Sebumetry evaluation</li> <li>• Scalp Hydration Exploratory Evaluation</li> <li>• Scalp skin Barrier Evaluation</li> <li>• Scalp skin pH Evaluation</li> <li>• Scalp Residue Assessment</li> <li>• Standardized Scalp Image Capture</li> <li>• Quality-of-Life Questionnaire</li> </ul> <p>At-Home Product Use:</p> <p>The application of the IPs was performed or supervised by the subjects' parents/LAR at home.</p> <p>The product kit was used for 28 ± 2 days, at least 3 times a week, totaling at least 12 uses of the complete routine (Shampoo, Conditioner, and Combing Cream).</p> <p>Visit 2 – Day 28:</p> <ul style="list-style-type: none"> <li>• Final Clinical Assessment</li> <li>• Scalp Microbiome Collection</li> <li>• Scalp Sebumetry evaluation</li> <li>• Scalp Hydration Exploratory Evaluation</li> <li>• Scalp skin Barrier Evaluation</li> <li>• Scalp skin pH Evaluation</li> <li>• Scalp Residue Assessment</li> <li>• Standardized Scalp Image Capture</li> <li>• Quality-of-Life Questionnaire</li> </ul>

<b>MEASUREMENT AND/OR EVALUATION SCHEDULE</b>	<table border="1"> <thead> <tr> <th rowspan="2">Protocol Activities</th> <th>Visit 1</th> <th rowspan="2">IPs usage at home and Subject's Diary completion*</th> <th>Visit 2</th> </tr> <tr> <th>Day 1</th> <th>Day 28**</th> </tr> </thead> <tbody> <tr> <td>Study Instructions and Signature of ICD and ICID</td> <td>X</td> <td>-</td> <td>-</td> </tr> <tr> <td>Eligibility Check (Review of inclusion/exclusion criteria)</td> <td>X</td> <td>-</td> <td>X</td> </tr> <tr> <td>Clinical Assessment (Dermatologist and Pediatrician)</td> <td>X</td> <td>-</td> <td>X</td> </tr> <tr> <td>Collection of 2 samples of the scalp microbiome through a non-invasive method</td> <td>X</td> <td>-</td> <td>X</td> </tr> <tr> <td>Instrumental measurements of scalp sebumetry</td> <td>X</td> <td>-</td> <td>X</td> </tr> <tr> <td>Instrumental measures of scalp hydration</td> <td>X</td> <td>-</td> <td>X</td> </tr> <tr> <td>Instrumental measures of scalp skin barrier</td> <td>X</td> <td>-</td> <td>X</td> </tr> <tr> <td>Instrumental measures of scalp pHmetry</td> <td>X</td> <td>-</td> <td>X</td> </tr> <tr> <td>Scalp residue assessment</td> <td>X</td> <td>-</td> <td>X</td> </tr> <tr> <td>Standardized Scalp Image Capture for Residue Registration (Photofinder)</td> <td>X</td> <td>-</td> <td>X</td> </tr> <tr> <td>Quality of Life Questionnaire</td> <td>X</td> <td>-</td> <td>X</td> </tr> <tr> <td>Distribution: - Investigational Products Kit - Subject Diary</td> <td>X</td> <td>-</td> <td>-</td> </tr> <tr> <td>Verbal instructions to subjects for completing the Diary and IPs usage</td> <td>X</td> <td>-</td> <td>-</td> </tr> <tr> <td>Collect diaries and IPs</td> <td>-</td> <td>-</td> <td>X</td> </tr> <tr> <td>Subject Diary Verification</td> <td>-</td> <td>-</td> <td>X</td> </tr> <tr> <td>AE/SAE assessment</td> <td>-</td> <td>X</td> <td>X</td> </tr> <tr> <td>Concomitant Medications Verification</td> <td>X</td> <td>-</td> <td>X</td> </tr> </tbody> </table>	Protocol Activities	Visit 1	IPs usage at home and Subject's Diary completion*	Visit 2	Day 1	Day 28**	Study Instructions and Signature of ICD and ICID	X	-	-	Eligibility Check (Review of inclusion/exclusion criteria)	X	-	X	Clinical Assessment (Dermatologist and Pediatrician)	X	-	X	Collection of 2 samples of the scalp microbiome through a non-invasive method	X	-	X	Instrumental measurements of scalp sebumetry	X	-	X	Instrumental measures of scalp hydration	X	-	X	Instrumental measures of scalp skin barrier	X	-	X	Instrumental measures of scalp pHmetry	X	-	X	Scalp residue assessment	X	-	X	Standardized Scalp Image Capture for Residue Registration (Photofinder)	X	-	X	Quality of Life Questionnaire	X	-	X	Distribution: - Investigational Products Kit - Subject Diary	X	-	-	Verbal instructions to subjects for completing the Diary and IPs usage	X	-	-	Collect diaries and IPs	-	-	X	Subject Diary Verification	-	-	X	AE/SAE assessment	-	X	X	Concomitant Medications Verification	X	-	X
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<b>INSTITUTIONAL REVIEW BOARD (IRB)</b>	<p>This study was reviewed and approved by the following IRB/IEC: - Investiga – Institutos de Pesquisa - Approval date: 14-Oct-2021</p>																																																																										
<b>SAFETY AND ADVERSE EVENTS</b>	<p>All Adverse Events (AEs/SAEs) were collected regardless of causal relationship to the subject's participation in the study. The information was collected/reported within the reporting timelines specified in the protocol. The conclusion on the IP's safety for the study population was issued by the Study Physician based on the study safety results presented in this summary clinical study report.</p>																																																																										

<p><b>MONITORING, QUALITY CONTROL, AND QUALITY ASSURANCE</b></p>	<p>The study monitoring was conducted as per the Sponsor’s requirements. The Study Site was/is subject to review by the IRB, to quality assurance audits performed by the Sponsor, and/or to inspection by appropriate regulatory authorities.</p>
<p><b>OVERALL CONCLUSIONS</b></p>	<p>Safety: Overall results from this study indicated that the products were safe under the conditions of use.</p> <p>Dermatological and Pediatric Clinical Assessments: A statistically significant improvement of desquamation was observed after 28 days of products use. No subjects presented Itching, Stinging, Burning, Irritation, Dryness and Erythema after 28 days of products use.</p> <p>Efficacy: No significant difference was observed on oiliness after 28 days of products use, showing scalp health maintenance. No significant improvement on scalp hydration was observed after 28 days of products use, showing scalp health maintenance. No significant improvement of scalp skin barrier was observed after 28 days of products use, showing scalp health maintenance. No significant change on scalp pH was observed after 28 days of products use, showing scalp health maintenance. A significant reduction on scalp residue was observed after 28 days of product use, indicating improvement on desquamation and false dandruff residues.</p> <p>Quality-of-Life Questionnaire: Descriptive statistical analysis of parents/LAR (Legally Acceptable Representative) responses to statements presented after 28 days of products use was performed. There were significant improvements in satisfaction with appearance of child’s hair (12.5%), curl maintenance (21.9%), reduction of "false dandruff" or "white masses" on child's scalp (9.4%) and less pain during the hair untangling (24.9%).</p> <p>Scalp Microbiome Collection: The main species that comprise the scalp microbiome were preserved in the subjects. There was a statistical decrease in readings for species of the genus <i>Staphylococcus</i>, and for <i>Malassezia</i>. Also, the <i>Cutibacterium acne</i> population was preserved. The decrease of <i>Malassezia globosa</i> presented a positive correlation for the TEWL and for the decrease of desquamation and false dandruff residues. The stability of the genus <i>Cutibacterium</i> after 28 days of products use is important for the maintenance of a health microbial community <sup>2</sup> and the stability of the species <i>C. acnes</i> has a positive correlation with health skin barrier. The decrease of <i>Staphylococcus</i> species showed a positive correlation with desquamation and false dandruff residues, except for <i>S. caprae</i> that increased and showed a negative correlation. There was no significant difference in the increase or decrease of alpha diversity (Shannon Index) between samples collected at D1 and after D28 days of product use, neither in Fungi nor in Bacteria; indicating maintenance of scalp microbiome diversity.</p>

**BIBLIOGRAPHIC REFERENCES**

- 1) SAXENA et al. Longitudinal study of the scalp microbiome suggests coconut oil to enrich healthy scalp commensals. Nature Portfolio: Scientific Reports. (2021) 11:7220.
- 2) RONG TAO, RUOYU LI, RUOJUN WANG. Skin microbiome alterations in seborrheic dermatitis and dandruff: A systematic review. Experimental Dermatology. (2021); 30:1546–1553.

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