Color Protection Assay-Microscopy Imaging

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Tradename: AC Det'Ox Hair

Code: 21030

CAS #: 8013-01-2 & 68333-16-4 (or) 92128-79-5

Test Request Form #: 13108

Lot #: N240115A

Sponsor: *Active Concepts, LLC – 107 Technology Drive, Lincolnton, North Carolina 28092*
Study Director: *Daniel Shill*
Principal Investigator: *Hannah Stade*

Test Performed:
Color Protection Assay-Microscopy Imaging

Introduction

Hair is colored for various reasons leading to several different tones, shades, and types of dye available for consumers. However, colored hair is prone to rapid fading and most color protection products do not live up to expectation. These products typically optimize a shampoo surfactant system to reduce the harsh cleansing and subsequent dye diffusion out of the hair shaft, or coat the hair with polymers, silicones, actives, or add a UV filter. Oxidative hair dyes permanently change hair color, however permanently dyed hair is susceptible to color fade and damage via wash-out, UV irradiation, and heat styling appliances. These cause shifts in the dyed-hair color tone resulting in dull, flat, and brassy hair.

Accordingly, an *ex vivo* Color Protection Assay was performed to determine qualitative and quantitative color fade benefits of **AC Det'Ox Hair** on hair.

Assay Principle

Blonde human hair tresses were dyed red as this shade shows the greatest level of damage from wash-out and UV irradiation. Tresses were treated with test materials for a determined number of wash cycles followed by UV exposure. Throughout the experiment, microscopy images were taken and color intensity (CIEL*a*b* values) was monitored to calculate color differences (ΔE).

Materials

A. **Hair Sample:** Blonde Virgin Human Hair Tresses Dyed Red
B. **Product:** Base Shampoo and Conditioner (Table 1)
C. **Equipment:** Accuris UV Transilluminator (25.3 W/m^2); DermaLab Skin Combo (Colori Probe and DermaScope Camera Probe)
D. **Software:** Excel Analysis ToolPak (Microsoft)

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Table 1. Base Shampoo and Base Conditioner Compositional Breakdowns

Base Shampoo Formulation	
INCI	**%**
Water	41.0
Guar Hydroxypropyltrimonium Chloride	1.0
Sodium Methyl 2-Sulfolaurate (and) Disodium 2-Sulfolaurate	35.0
Cocamidopropyl Betaine	15.0
Lactobacillus Ferment & Lactobacillus & Cocos Nucifera (Coconut) Fruit Extract	4.0
Polysorbate 20	2.0
Fragrance	2.0

Base Conditioner Formulation	
INCI	**%**
Water	76.0
Polyquaternium-10	1.0
Glycerin	3.0
Water & Centrimonium Chloride	2.0
Behentrimonium Methosulfate & Cetearyl Alcohol & Butylene Glycol	8.0
Hydrogenated Ethylhexyl Olivate (and) Hydrogenated Olive Oil Unsaponifiables	5.0
Lactobacillus Ferment	4.0
Fragrance	1.0

Methods

Blonde hair tresses were dyed red as this is the shade which shows the greatest level of wash-out. Four tresses were assigned to each condition described in Table 2. Eight tresses were treated with Base Shampoo and Conditioner, eight with 2.0% **AC Det'Ox Hair** in base shampoo and conditioner, and four were left as Untreated Controls. The study was conducted using a blind protocol.

The dyed hair tresses were washed, and air dried 7 or 14 times dependent on conditions and associated treatments (Table 2). Color intensity (L*a*b*) was evaluated for each tress before washing, after 7 washes, and after 14 washes.

The tresses were exposed to 0, 10, 20, or 30 hours of UV irradiation using a UV lamp at an irradiation intensity of 25.259 W/m^2. Microscopic examination of the hair tresses was conducted utilizing the DermaLab Skin Combo DermaScope Camera Probe. Additionally, color intensity was evaluated after each UV exposure time.

Table 2. Descriptions of the Conditions and Treatments for each Set of Hair Tresses.

Condition	Treatment Description
Untreated Control	0 Washes; 0, 10, 20, 30 Hours UV Exposure
Base Shampoo and Conditioner	7 Washes; 0, 10, 20, 30 Hours UV Exposure
Base Shampoo and Conditioner	14 Washes; 0, 10, 20, 30 Hours UV Exposure
2.0% **AC Det'Ox Hair** in Base Shampoo and Conditioner	7 Washes; 0, 10, 20, 30 Hours UV Exposure
2.0% **AC Det'Ox Hair** in Base Shampoo and Conditioner	14 Washes; 0, 10, 20, 30 Hours UV Exposure

Average color intensity (L*a*b*) values for tresses within each condition were calculated. Color difference (ΔE) is measured on a scale from 0.00 to 100.00 where 0.00 is no difference in color and 100.00 is complete color distortion (Table 3) and calculated for each condition and time point using the following equation:

$$Color\ Difference\ (\Delta E) = \sqrt{(L^*_{Treatment} - L^*_{Baseline})^2 + (a^*_{Treatment} - a^*_{Baseline})^2 + (b^*_{Treatment} - b^*_{Baseline})^2}$$

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Table 3. Standard perception ranges to assess changes in color (ΔE values)

ΔE Value	Visual Perception
≤ 1.00	Not perceivable
1.00-2.00	Perceivable through close observation
2.00-10.00	Perceivable at glace
11.00-49.00	Colors are more similar than opposite
> 49.00-100.00	Colors are more opposite than similar or exactly opposite

Data are displayed as averages and analyzed using a one-way ANOVA with statistical significance accepted at $p \leq 0.05$.

Results

The data obtained from this study met criteria for a valid assay and the Untreated Control performed as anticipated. Compared to the Untreated Control, the tresses treated with the Base Shampoo and Conditioner demonstrated significant color loss after both 7 and 14 washes which was further exacerbated by cumulative UV exposure. The tresses treated with 2.0% **AC Det'Ox Hair** in base shampoo and conditioner provided color protection and reduced color fade compared to the Base Shampoo and Conditioner.

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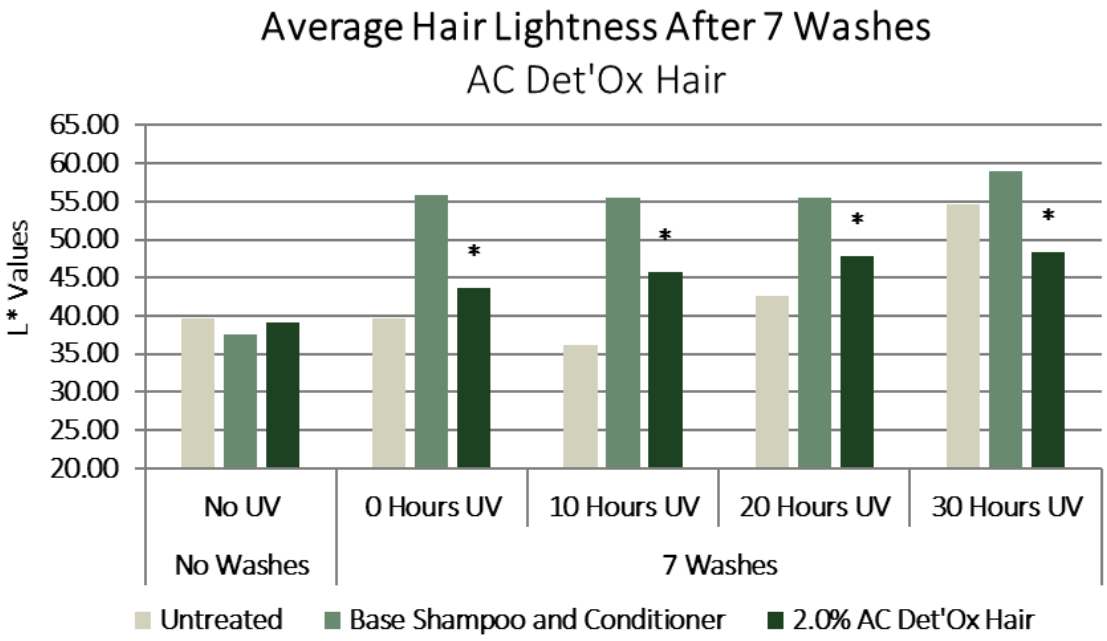

Figure 1. Average Hair Color Lightness (L*) After 7 Washes with and without UV Exposure. * indicates significance ($p \leq 0.05$) compared to Base Shampoo and Conditioner. Base Shampoo and Conditioner was significantly different compared to the Untreated at all time points after Baseline. Baseline: hair tresses without washes and no UV exposure.

Table 4. Results from one-way ANOVA Statistical Analysis of Color Lightness (L*) for Hair Tresses Washed 7 Times at Each Time Point. * indicates significance ($p \leq 0.05$) between the two conditions compared.

	Baseline	After 7 Washes, 0 Hours UV	After 7 Washes, 10 Hours UV	After 7 Washes, 20 Hours UV	After 7 Washes, 30 Hours UV
Untreated vs Base Shampoo and Conditioner	> 0.05	0.009*	0.045*	0.005*	0.016*
Untreated vs 2.0% **AC Det'Ox Hair**	> 0.05	> 0.05	> 0.05	0.001*	> 0.05
Base Shampoo and Conditioner vs 2.0% **AC Det'Ox Hair**	> 0.05	0.015*	0.009*	0.032*	< 0.001*

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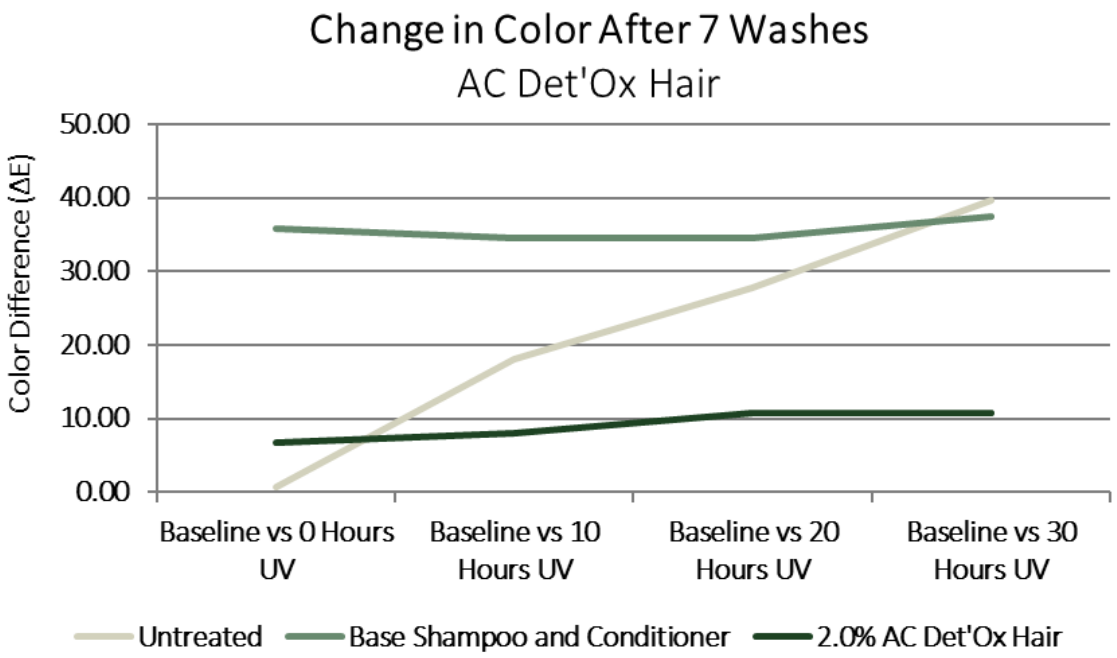

Figure 2. Perceivable Color Difference (ΔE) After 7 Washes with and without UV Exposure, where 0.00 represents no change and 100.00 represents complete color distortion. Baseline: hair tresses without washes and no UV exposure.

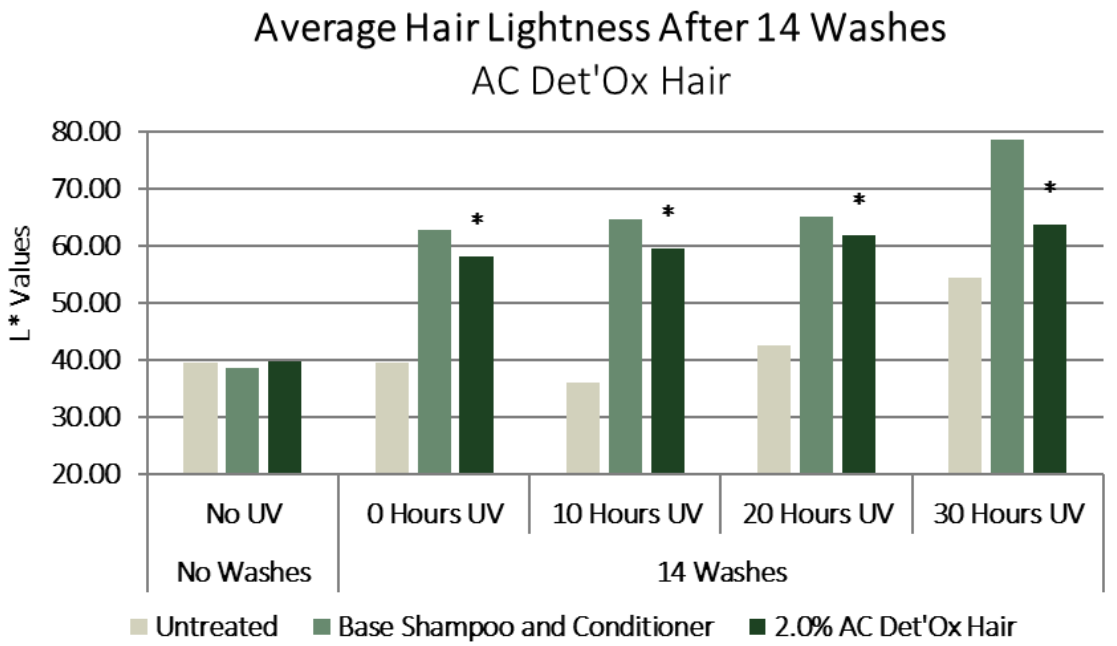

Figure 3. Average Hair Color Lightness (L*) After 14 Washes with and without UV Exposure. * indicates significance ($p \leq 0.05$) compared to Base Shampoo and Conditioner. Base Shampoo and Conditioner and 2.0% **AC Det'Ox Hair** were significantly different compared to the Untreated at all time points after Baseline. Baseline: hair tresses without washes and no UV exposure.

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Table 5. Results from one-way ANOVA Statistical Analysis of Color Lightness (L*) for Hair Tresses Washed 14 Times at Each Time Point. * indicates significance ($p \leq 0.05$) between the two conditions compared.

	Baseline	After 14 Washes, 0 Hours UV	After 14 Washes, 10 Hours UV	After 14 Washes, 20 Hours UV	After 14 Washes, 30 Hours UV
Untreated vs Base Shampoo and Conditioner	> 0.05	0.013*	0.020*	< 0.001*	0.010*
Untreated vs 2.0% AC Det'Ox Hair	> 0.05	0.019*	0.041*	0.002*	0.010*
Base Shampoo and Conditioner vs 2.0% AC Det'Ox Hair	> 0.05	0.044*	0.007*	0.025*	0.027*

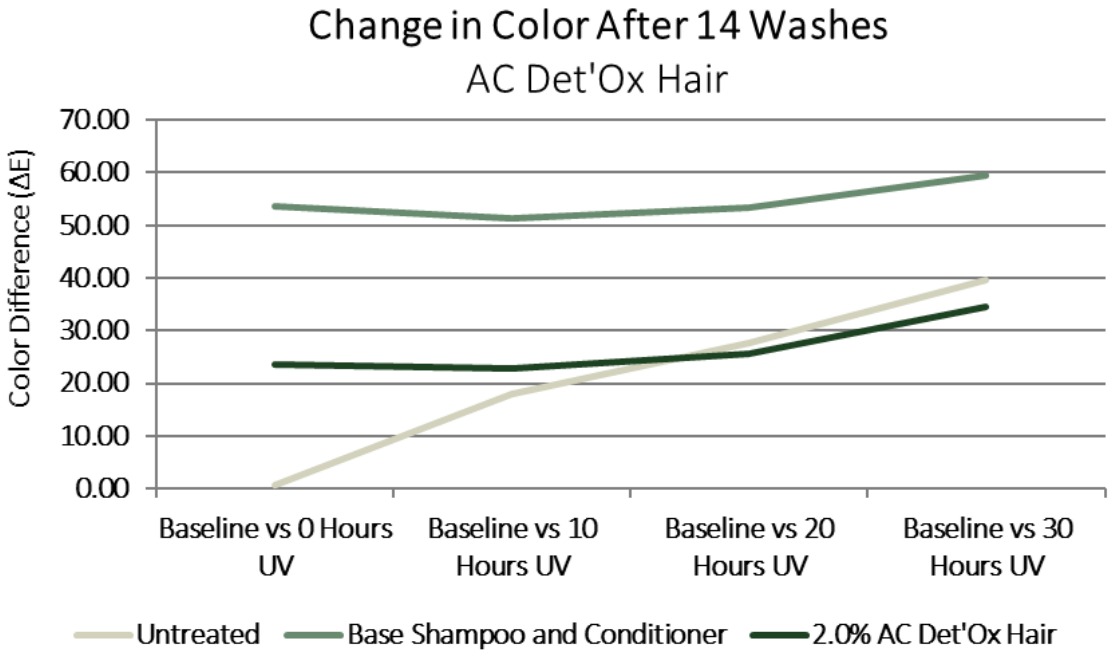

Figure 4. Perceivable Color Difference (ΔE) After 14 Washes with and without UV Exposure, where 0.00 represents no change and 100.00 represents complete color distortion. Baseline: hair tresses without washes and no UV exposure.

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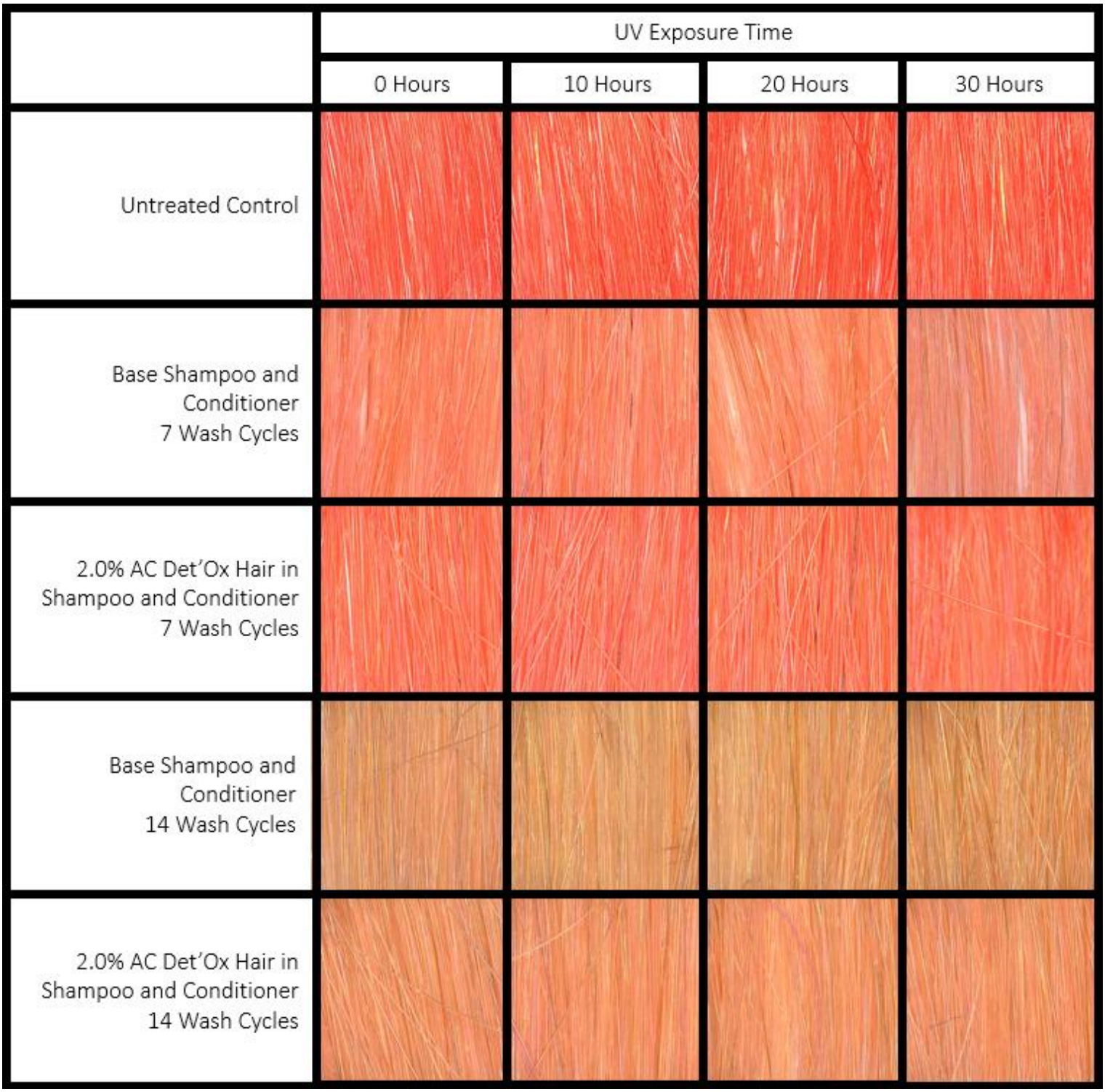

Figure 5. Representative Microscopy Images of Color-Fade Demonstrating the Influence of UV Exposure and Number of Wash Cycles on Hair Colorfastness.

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Discussion

A color protection assay was performed to determine the color-fade benefits of AC Det'Ox Hair on hair. The results from this study indicate 2.0% AC Det'Ox Hair provides color protection against repeated washes and UV exposure.

As shown in Figures 1, 3, and 5, the Untreated Control hair tresses demonstrated an increase in color lightness of 38% after 30 hours of UV exposure indicative of color fade. Furthermore, after 30 hours of UV exposure the Untreated Control hair had a perceivable color difference of 37.73 (Figures 2 and 4). These data indicate UV exposure exacerbates color loss in hair.

Hair washed 7 times with Base Shampoo and Conditioner elicited a 49% increase in color lightness compared to baseline. Hair color lightened further by 57% after exposure to 30 hours of UV irradiation compared to baseline (Figure 1). Additionally, the color difference after 7 washes with Base Shampoo and Conditioner was 35.82 and reached a max of 37.41 after 30 hours of UV exposure compared to baseline (Figure 2). Color loss in tresses washed 7 times with Base Shampoo and Conditioner was significantly higher than the Untreated Control tresses at all time points after baseline (Table 4). These data indicate washing hair 7 times accelerates hair color fade which is further exacerbated by UV exposure in a dose-dependent fashion.

Conversely, hair tresses washed 7 times with 2.0% AC Det'Ox Hair only elicited a 12% increase in color lightness compared to baseline. Hair continued to lighten by 24% after 30 hours of UV exposure compared to baseline (Figure 1). Furthermore, the color difference after 7 washes with 2.0% AC Det'Ox Hair was 6.82 and only reached a max of 10.76 after 30 hours of UV exposure compared to baseline (Figure 2). Color lightening of tresses treated with 2.0% AC Det'Ox Hair was significantly less than the Base Shampoo and Conditioner after 7 washes with and without UV exposure at all durations (Table 4). These data indicate 2.0% AC Det'Ox Hair blunts the hair color fade process after 7 washes and UV exposure compared to the Base Shampoo and Conditioner alone.

Hair tresses washed 14 times with Base Shampoo and Conditioner experienced dramatic color loss denoted by an increase of 63% in color lightness and a color difference of 53.75 compared to baseline. After exposure to 30 hours of UV irradiation, color lightness increased by 104% and reached a max color difference of 59.37 after 30 hours of UV irradiation compared to baseline (Figures 3 and 4). Color loss in tresses washed 14 times with Base Shampoo and Conditioner was significantly higher than the Untreated Control hair tresses at all time points after baseline (Table 5). These data indicate washing hair 14 times accelerates hair color fade which is further exacerbated by UV exposure in a dose-dependent fashion.

Hair tresses washed 14 times with 2.0% AC Det'Ox Hair experienced dramatic color loss but to a lesser extent than the Base Shampoo and Conditioner. After washing with 2.0% AC Det'Ox Hair, color lightness increased by 49% and color difference compared to baseline was 23.53. After exposure to 30 hours of UV irradiation, color lightness increased by 60% and reached a max color difference of 34.40 after 30 hours of UV irradiation compared to baseline (Figures 3 and 4). Color lightening in tresses washed 14 times with 2.0% AC Det'Ox Hair and exposed to UV irradiation was significantly less compared to Base Shampoo and Conditioner at all exposure durations (Table 5). These data indicate 2.0% AC Det'Ox Hair mitigates the hair color fade process after 14 washes and UV exposure compared to the Base Shampoo and Conditioner alone.

Taken together, these qualitative and quantitative results indicate AC Det'Ox Hair attenuates the loss in color intensity and color difference in dyed hair associated with repeated washing and UV exposure when added to personal care applications at recommended use levels. Collectively, AC Det'Ox Hair demonstrates a protective effect against the color fade process which improves hair health and contributes to the appearance of more vibrant and brighter dyed hair for a longer period of time.