

## **Evaluation of Product pH and Determination of Peroxide Release in Saliva: Chairside, Paint-On Application of WSD Hybrid Teeth Whitening Gel Containing 35% Carbamide Peroxide Equivalent**

*Martin Giniger, DMD, MsD, PhD, FICD*

Chief Investigator, PSC Research Institute\*, Delray Beach Florida 33445

\*PSC Research Institute is a GLP-compliant laboratory

Dates of Study: September 23 to September 24, 2016

### **Abstract:**

The aim of the study was to determine the salivary concentration of peroxide released during the chairside, paint-on application of a teeth whitening gel containing a 35% Carbamide Peroxide equivalent. This “hybrid” gel was manufactured by WSD Labs USA LLC. The pH of the final admixed product was also determined just prior to application onto teeth following the OECD Draft Test Method for measuring pH, Acidity and Alkalinity.

For determination of salivary concentration of peroxide, subjects maxillary and mandibular teeth were bleached using a syringe filled with 500 mg of gel comprised of 14% Carbamide Peroxide and 7% Hydrogen Peroxide. The gel was painted onto the teeth using a micro paintbrush and exposed to a 24 watt LED lamp for 2 sessions of 20 minutes each (40 minutes total exposure time).

Saliva was collected before and during the whole period of bleaching at different intervals (immediately after application, and at 30 seconds, 5 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes, 40 minutes, 60 minutes and 8 hours after the product was initially applied.). The amount of hydrogen peroxide in the salivary samples was assessed with peroxidase, phenol and 4-aminoantipyrin in a photometric assay. Additionally the amount of peroxides in the bleaching material was determined before the bleaching, so that percentages could be calculated. Assessments of oral hard and soft tissues were also performed at all measurement intervals by a trained oral pathologist.

### **Results:**

The pH of the gel was determined to be  $5.91 \pm 0.05$ , and this was well above the pH minimum (pH must be above 4.0) established by the Canadian Food and Drug Administration. The amount of peroxides released into saliva was also well within safety guidelines (does not exceed 3%). In fact, we found that at no time did the level rise above 0.62% that was seen at 25 minutes after the initial application of gel (5 minutes after the 2<sup>nd</sup> application). The percentages at other time points were all below 0.60% or less. After the gel was rinsed off of teeth were removed at the 40-minute mark, the peroxide levels quickly returned towards baseline as evidenced by the low amount at the 60-minute measurement (0.09%). At 8 hours, the salivary concentration of Hydrogen Peroxide returned to zero (0.0mg).

Furthermore, there were no ill effects caused by the test product in all ten (10) subjects studied. There were no adverse reactions and no tissue changes found as assessed by a trained oral pathologist.

### **Conclusions:**

It can be concluded that professionally applied, WSD Labs USA hybrid teeth whitening gel containing 14% Carbamide Peroxide and 7% Hydrogen Peroxide is safe to use as its properties are well within the guidelines established in section 16 of the *Canadian Food and Drugs Act*.

### **Methods:**

Ten subjects were medically fit and healthy, aged between 18 and 68 years, had a good standard of oral hygiene and a minimum of 20 permanent, caries-free teeth. They each gave informed consent to participation in the investigation, which had ethical approval.

### **Product Description:**

The WSD Hybrid Teeth Whitening Gel containing 35% Carbamide Peroxide Equivalent comprises a combination of 14% Carbamide Peroxide and 7% Hydrogen Peroxide, and is intended to be used in-office teeth whitening system. The gel is contained within a syringe and the gel is applied directly onto teeth using a micro paintbrush for two 20-minute cycles (total of 40 minutes) using the following method:

1. Place protective eyewear and bib on patient and apply petroleum jelly to moisten their lips.
2. Place plastic cheek retractors. Insert the cheek retractor in the patient's mouth, retracting one side at a time.
3. Completely rinse and air-dry teeth.
4. Apply vitamin E capsule to tissue surrounding teeth, with a micro-brush to prevent blanching, going one tooth beyond the most distal tooth that is being whitened.
5. Twist off protective cap from Whitening gel syringe tip. Using a micro-brush, paint 250 mg of Hybrid Teeth Whitening Gel directly to the facial surface of the teeth, avoiding all soft tissue. If the patient has any crowns in their mouth, and do not apply whitening gel to those areas.
6. Insert saliva ejector and raise the chair slightly so the patient is comfortable and able to swallow.
7. Place protective eye wear on patient and align the standard 24 watt, professional L.E.D. bleaching lamp so that the light is close to the teeth without touching. Press the Power button on the light and make sure the timer is set for twenty (20) minutes.
8. After the first 20 minute cycle is done, carefully and thoroughly remove the gel from the teeth with a surgical suction tip. Re-apply the vitamin E to the surrounding tissue and follow steps 5-6.
9. After the second and final cycle is complete, remove the light and carefully and thoroughly remove the gel with a surgical suction tip.
10. Remove protective eye wear and carefully remove the cheek retractor and have the patient thoroughly rinse their mouth with water.

In total, we carefully applied 500 mg of the bleaching gel (2 x 250mg) according to manufacturers instructions above. Using the measurement technique described below, each 500 mg of gel contained 58.5 mg of hydrogen peroxide (11.7%).

### **Experimental Plan:**

Subjects who met inclusion / exclusion criteria from a pool of subjects in south Florida were treated with the test product by a licensed dentist / pathologist according to manufacturers instructions. The following measures of safety and efficacy were collected at each examination interval:

- Oral Hard & Soft Tissue Evaluation by trained oral pathologist
- Assessment for Adverse Reactions by trained oral pathologist
- Periodic saliva collection followed by assay for Hydrogen Peroxide concentration by GLP-compliant laboratory

The study followed a double-blind research protocol that complied with industry and ADA standards. Full details for all study procedures are contained within the approved study protocol,

### **pH Determination:**

The pH of the final admixed product was determined just prior to application onto teeth following the OECD Draft Test Method for pH, Acidity and Alkalinity. The method is described at <http://www.oecd.org/dataoecd/27/63/49070189.pdf>. The pH of the aqueous formula was determined with a standard pH meter equipped with an appropriate contact-read electrode system. The acidity or alkalinity of the formulation in water is determined by titration with standard acid or alkali using electrometric end-point detection.

### **Collecting of saliva samples:**

Bleaching with the WSD 35% Carbamide Peroxide Equivalent teeth whitening gel began two hours after tooth brushing without eating and drinking in the meantime. Before bleaching was performed, an un-stimulated saliva sample (2 ml) was taken for the determination of the reagent blank value. Immediately after beginning of bleaching, the first saliva sample was taken after 30 seconds. Further samples were collected in different cups analyzed after 30 seconds, 5 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes, 40 minutes, 60 minutes and 8 hours. A final sample was taken 8 hours after the product was applied.

### **Determination of peroxides:**

A photometric method modified according to Bauminger<sup>1</sup> was used for determination of peroxides in bleaching agents and salivary samples. The method is based on the reaction of 4-aminoantipyrin and phenol with H<sub>2</sub>O<sub>2</sub> catalyzed by peroxidase. It is known, that organic peroxides are oxidized by peroxidase. Thereby oxygen is released which subsequently oxidizes achromatic chromogenic hydrogen donors. The reagents were stored at 4°C until used in the experiment. Twenty microliter of saliva were added to 1000 µl enzyme reagent. Directly afterwards, the absorbance was measured at  $\lambda=510$  nm against reagent blank (saliva sample free of peroxides). The peroxide concentration was calculated according to the Lambert-Beer's law.

### **Bleaching procedures :**

Eight (8) Maxillary and eight (8) Mandibular teeth were bleached in accordance manufacturers instructions described above.

### **Statistics:**

Due to the lack of normal distribution, statistical analysis was performed with *Students T-test* Significance was set at  $P \leq 0.05$ .

<sup>1</sup> Bauminger, B. *J Clin Pathol* 1974; 27:1015-1017

## **RESULTS:**

**DEMOGRAPHICS**

All subjects were residents of South Florida and all major races were represented in the study groups. All subjects were healthy and had healthy, natural anterior teeth. The mean age was  $47.4 \pm 16.2$ . There were 5 females and 5 males that participated in the study.

**ORAL EXMINATIONS – GENERAL SAFETY**

We found that there were no ill effects caused by the test product in all ten (10) subjects studied. There were no adverse reactions and no tissue changes found except for mild reversible blanching found in only one of the subjects. A suitably trained specialist in Oral Pathology examined all subjects at each examination interval and found no clinical irritations or pathologies on the oral hard / soft tissues.

**PH DETERMINATION**

The pH of the bleaching gel was determined to be  $\text{pH} = 5.91 \pm 0.05$ . This measurement is an average of triplicate readings (see Table 1).

Table 1 – Triplicate pH Determination

	pH
Reading 1	5.95
Reading 2	5.86
Reading 3	5.91

**SALIVARY HYDROGEN PEROXIDE LEVELS**

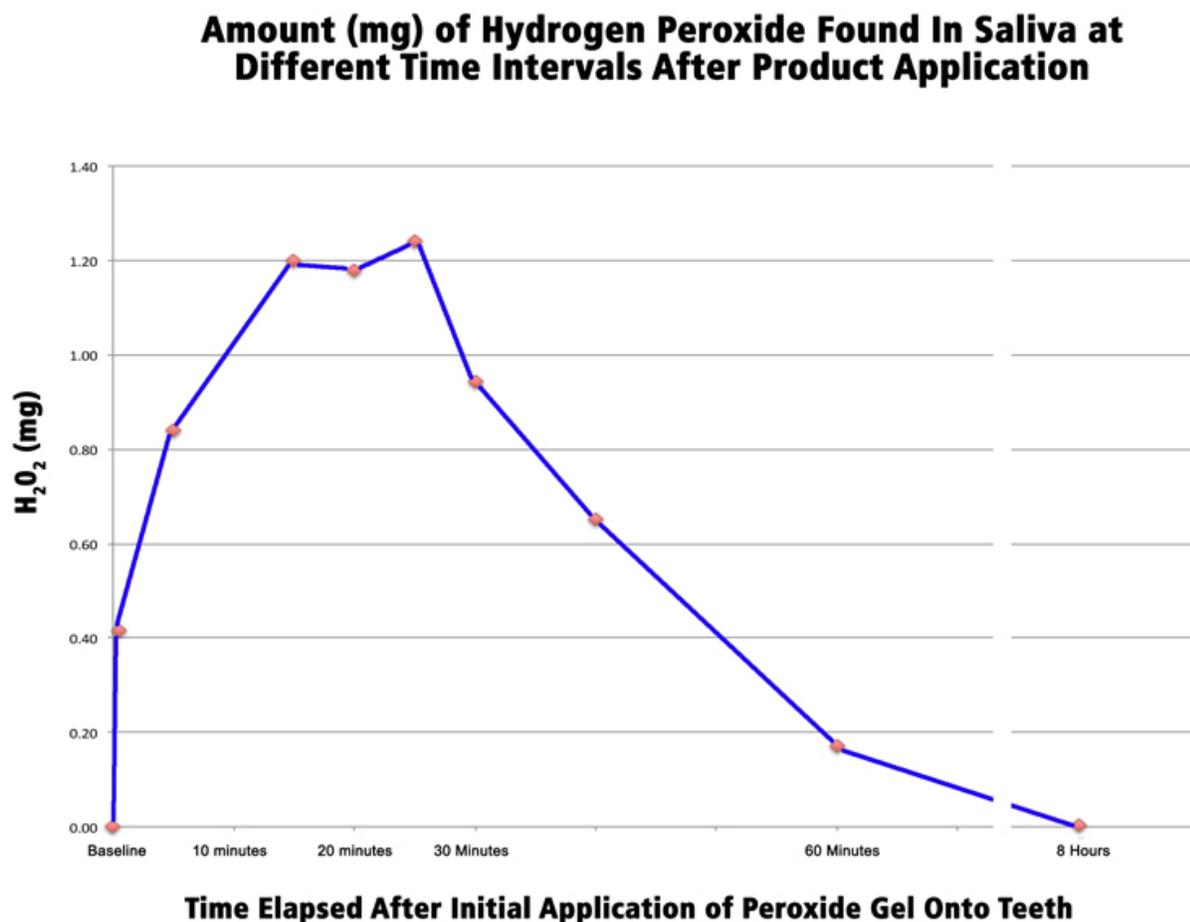
Hydrogen peroxide release into the oral cavity reached its maximum in the first 25 minutes ( $1.24 \text{ mg} \pm 0.19$ ; 0.62%) and this was well below the 3% threshold set by Canadian statutes. At 30 seconds (almost immediately after product application we recovered  $0.42 \text{ mg} \pm 0.07$ ; or 0.21%). We were also able to measure Hydrogen peroxide in saliva at 5, 15, 20, 25, 30, 40 and 60 minutes. At 60 minutes we found only small amounts of peroxides were released (less than 0.1%) into saliva. At eight (8) hours the salivary concentration of Hydrogen Peroxide returned to baseline levels (0.00 mg; 0.00%). The amount and percent concentrations of hydrogen peroxide found in salivary samples of the ten (10) subjects are shown in Table 2 and Figure 1.

Table 2 – Amount of Hydrogen Peroxide Found in Saliva After Product Application

Time Elapsed	Amount of Hydrogen Peroxide (mg) in 200mg of Collected Saliva											
	1	2	3	4	5	6	7	8	9	10	MEAN (mg)	MEAN (PCT)
Before Treatment*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00%
30 Seconds	0.41	0.34	0.38	0.27	0.42	0.45	0.50	0.48	0.51	0.42	0.42	0.21%
5 Minutes	0.85	0.77	0.84	0.63	0.87	0.82	0.90	0.87	0.92	0.89	0.84	0.42%
15 Minutes	1.26	1.17	1.22	1.08	1.01	1.30	1.01	1.47	1.19	1.29	1.20	0.60%
20 Minutes	1.23	1.33	1.20	1.12	1.07	1.27	0.76	1.38	1.15	1.28	1.18	0.59%
25 Minutes	1.45	1.21	1.28	1.02	1.05	1.51	0.96	1.4	1.13	1.38	1.24	0.62%
30 Minutes	0.94	1.03	1.13	0.84	0.56	1.16	0.73	1.03	1.09	0.92	0.94	0.47%
40 Minutes	0.81	0.61	0.78	0.43	0.45	0.76	0.54	0.74	0.84	0.57	0.65	0.33%
60 Minutes	0.15	0.13	0.28	0.18	0.23	0.05	0.15	0.12	0.23	0.18	0.17	0.09%
8 Hours	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00%

\* Salivary Content of Hydrogen Peroxide Before Treatment Standardized and Set to 0.00mg

Figure 1 – Kinetics of Hydrogen Peroxide Release Into Saliva Over Time

**CONCLUSIONS:**

The aim of the study was to determine the salivary concentration of peroxide released during the chairside, paint-on application of a “hybrid” teeth whitening gel containing 35% Carbamide Peroxide equivalent. The pH of the gel was determined to be  $5.91 \pm 0.05$ , and this was well above the pH minimum (pH must be above 4.0) established by the Canadian Food and Drug Administration. The amount of peroxides released into saliva was also well within safety guidelines (does not exceed 3%). In fact, we found that at no time did the level rise above 0.62% that was seen at 25 minutes after the initial application of gel (5 minutes after the 2<sup>nd</sup> application). The percentages at other time points were all below 0.60% or less. After the gel was rinsed off of teeth were removed at the 40-minute mark, the peroxide levels quickly returned towards baseline as evidenced by the low amount at the 60 minute measurement (0.09%). At 8 hours, the salivary concentration of Hydrogen Peroxide returned to zero (0.0mg).

Furthermore, there were no ill effects caused by the test product in all ten (10) subjects studied. There were no adverse reactions and no tissue changes found as assessed by a trained oral pathologist. Therefore, it can be concluded that the WSD 35% Carbamide Peroxide Equivalent teeth whitening gel is safe to use as its properties are well within the guidelines established in section 16 of the *Canadian Food and Drugs Act*.