

Vital Medical Diagnostics Inc.

697 Front Road North, Amherstburg, Ontario N9V 2V6

Comparative Study of Three Commercially Available
Rapid Assays for the Detection of Occult Blood in Stool

Hemoccult (SmithKline Diagnostics, Inc.)
Hemoccult Sensa (SmithKline Diagnostics, Inc.)
Preview O.B.T. (Leeco Diagnostics, Inc.)

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Background and Introduction

The basic technology behind fecal occult blood testing, i.e., gum guaiac chemistry, has been known for over 20 years (1). Rapid diagnostic tests for occult blood in stool have been available for use in the physician's office and over-the-counter markets for nearly 10 years. The consensus market leader is SmithKline Diagnostics (San Jose, CA), whose Hemoccult product was originally developed by Laboratory Diagnostics, Inc., of Roselle, NJ. (now a subsidiary of Cenogenics, Inc.). The Hemoccult product has several years of solid clinical data supporting its use as a fecal occult screen. The "Hemoccult" name is virtually synonymous with occult blood testing.

An improved version of the product, Hemoccult Sensa, was recently introduced into the professional market. The rationale behind this product has been to increase the sensitivity of guaiac chemistry to more reliably detect abnormal bleeding associated with gastrointestinal disorders.

The following study was conducted to compare three brands of commercially available diagnostic assays for the detection of occult blood in stool. These tests are read qualitatively, and are used to screen for the presence of occult blood in feces, which has been widely documented to be an indicator of several gastrointestinal ailments including colorectal cancer (2,3).

The three brands utilized in the study were SmithKline's Hemoccult, SmithKline's Hemoccult Sensa, and the Preview O.B.T. brand manufactured for Leeco Diagnostics, Inc. (Southfield, MI). Leeco Diagnostics also obtains their product from Cenogenics, Inc. The study was performed to quantify the relative performance of the guaiac chemistry of the tests. All three utilize the same basic enzymatic chemistry reaction on guaiac paper which provides a colorimetric result in the presence of hemoglobin. Gum guaiac is a phenolic compound which has long been recognized as the most appropriate active ingredient in tests for fecal occult blood detection. The basic reaction involved in all gum-guaiac tests is the oxidation of the phenolic compounds. Peroxidases contained in the heme portion of the human hemoglobin in the fecal occult blood release oxygen molecules into solution upon the addition of developer (a stable solution of hydrogen peroxide and denatured ethanol). This oxidation results in a color change on the guaiac paper from a clear (white background) to a blue color. The presence of any blue color on the developed test paper is indicative of the presence of occult blood in stool, and patients are recommended to seek further confirmatory testing.

Materials and Methods

The comparison between the three brands of guaiac slides was made using 2X crystalline human hemoglobin (Sigma Chemical, St. Louis, MO). This material was reconstituted with distilled water to hemoglobin concentrations of 0.075 mg/ml, 0.02 mg/ml and 0.009 mg/ml. These concentrations were chosen because they represented values which tested both the upper ("positive" blue signal) and lower ("negative" clear result) limits of the guaiac paper of all three brands.

Once the hemoglobin was reconstituted, 20 microliters of each dilution was added to the two test areas on the front of each slide. Following the instructions of each kit, the front cover of the slide was closed and allowed to incubate at room temperature for 3 minutes. The slide was then flipped over and the back flap opened. Two drops of the respective developers were added to the guaiac paper over the area to which the sample had been added. Each brand has its own developing solution, and care was taken to insure that the appropriate developer was added to the corresponding slide. The results were visually evaluated by two technicians using a qualitative color intensity scale developed by SmithKline and which appears in its Hemocult Sensa brochure as follows:

| <u>Color Intensity</u> | <u>Description</u> |
|------------------------|---|
| - | No blue color |
| +1 | Very faint, barely detectable trace of blue |
| +2 | Faint blue color |
| +3 | Distinctly blue color |
| +4 | Intense blue. Wider area of blue color coverage more than a score of 3. |

Results were read to the nearest 0.5 on this scale of 0 to 4, and both a mean and standard deviation for each run were calculated. Developed slides of all three brands were read both immediately after the application of the developer, and also at 2 minutes following the application of the developer. This was done to demonstrate both the comparative strengths as well as the stability of the signals at each dilution.

The number of samples tested with each brand and by hemoglobin dilution are shown in Table 1.

TABLE 1

Number of Samples tested by Hemoglobin Concentration and Brand

| <u>Hemoglobin Concentration</u> | <u>Hemoccult</u> | <u>Hemoccult Sensa</u> | <u>Preview O.B.T.</u> |
|---------------------------------|------------------|------------------------|-----------------------|
| 0.075 mg/ml | 100 | 80 | 100 |
| 0.02 mg/ml | 68 | 100 | 100 |
| 0.009 mg/ml | <u>60</u> | <u>71</u> | <u>76</u> |
| TOTAL TESTED | 228 | 251 | 276 |

Results

The results of the comparative evaluation, read immediately and read at 2 minutes are shown in Tables 2 and 3, respectively. An average score and standard deviation were calculated for each brand and each dilution.

The results of the comparison indicate that the Hemoccult Sensa and Preview O.B.T. provide the user with a stronger blue signal than the standard Hemoccult test. This trend grows more apparent at increasingly higher dilutions. Additionally, both the Sensa and O.B.T. products provide a more consistent signal at higher dilutions, as shown by the size of the standard deviation relative to the mean. As the concentration drops to 0.009 mg/ml, the Hemoccult product when read immediately (Table 2) had a standard deviation greater than the mean, indicating wide variability in the results. The Sensa and O.B.T. products maintained standard deviations of approximately one half of the mean value.

The trends seen in Table 2 are accentuated in Table 3, which exhibits results of the same test slides after 2 minutes. While the results at 0.075 mg/ml (highest concentration used) are similar after 2 minutes, the differences become readily apparent at lower concentrations. Both the Sensa and O.B.T. products maintain similar intensity signals through the next two dilutions, while the Hemoccult provides virtually no signal (0.04 and 0.05, respectively).

TABLE 2

Comparative Results at Each Dilution - Read Immediately

Average Score and Standard Deviation

| <u>Hemoglobin Concentration</u> | <u>Hemoccult</u> | <u>Hemoccult Sensa</u> | <u>Preview O.B.T.</u> |
|---------------------------------|------------------|------------------------|-----------------------|
| 0.075 mg/ml | 3.2 ±0.31 | 3.44 ±0.33 | 3.5 ±0.0 |
| 0.02 mg/ml | 1.08 ±0.35 | 1.80 ±0.29 | 1.89 ±0.29 |
| 0.009 mg/ml | 0.21 ±0.25 | 0.75 ±0.3 | 0.53 ±0.19 |

TABLE 3

Comparative Results at Each Dilution - Read At 2 Minutes

Average Score and Standard Deviation

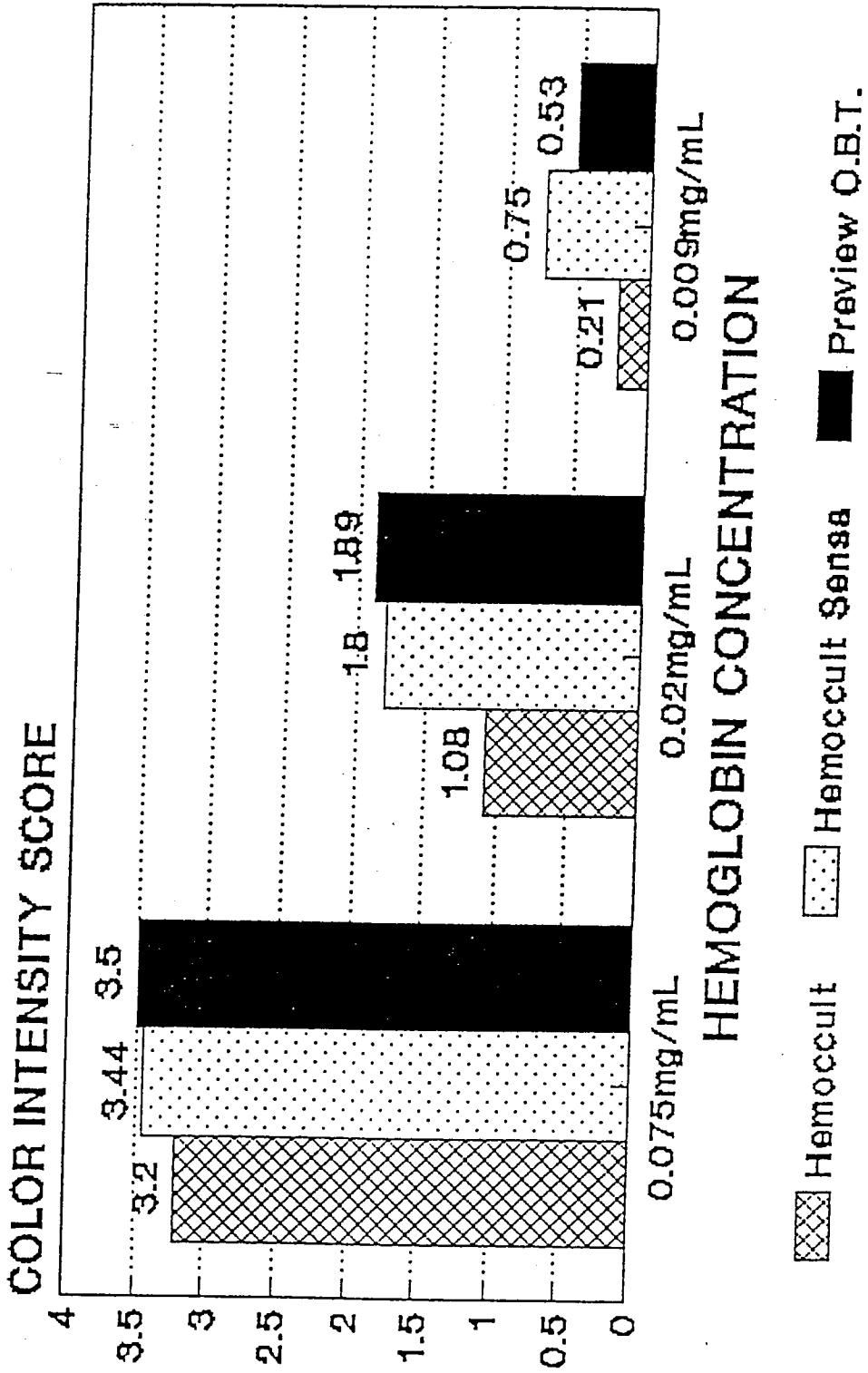
| <u>Hemoglobin Concentration</u> | <u>Hemoccult</u> | <u>Hemoccult Sensa</u> | <u>Preview O.B.T.</u> |
|---------------------------------|------------------|------------------------|-----------------------|
| 0.075 mg/ml | 2.7 ±0.35 | 3.1 ±0.22 | 3.3 ±0.25 |
| 0.02 mg/ml | 0.05 ±0.42 | 1.28 ±0.30 | 1.40 ±0.37 |
| 0.009 mg/ml | 0.04 ±0.12 | 0.30 ±0.23 | 0.22 ±0.21 |

Conclusions

This study was developed and conducted in an effort to demonstrate the relative strengths of the guaiac chemistries of the three brands of fecal occult blood tests. Based on the results of this comparison, it is clear that the signal intensity and stability of the Preview O.B.T. and the Hemoccult Sensa are virtually equal, while those of the Hemoccult are generally poorer. This is particularly true both at higher dilutions of hemoglobin, and after longer development times. The differences between the Sensa and O.B.T. products are marginal at best, with each product showing similar results at high hemoglobin dilutions and at longer development times.

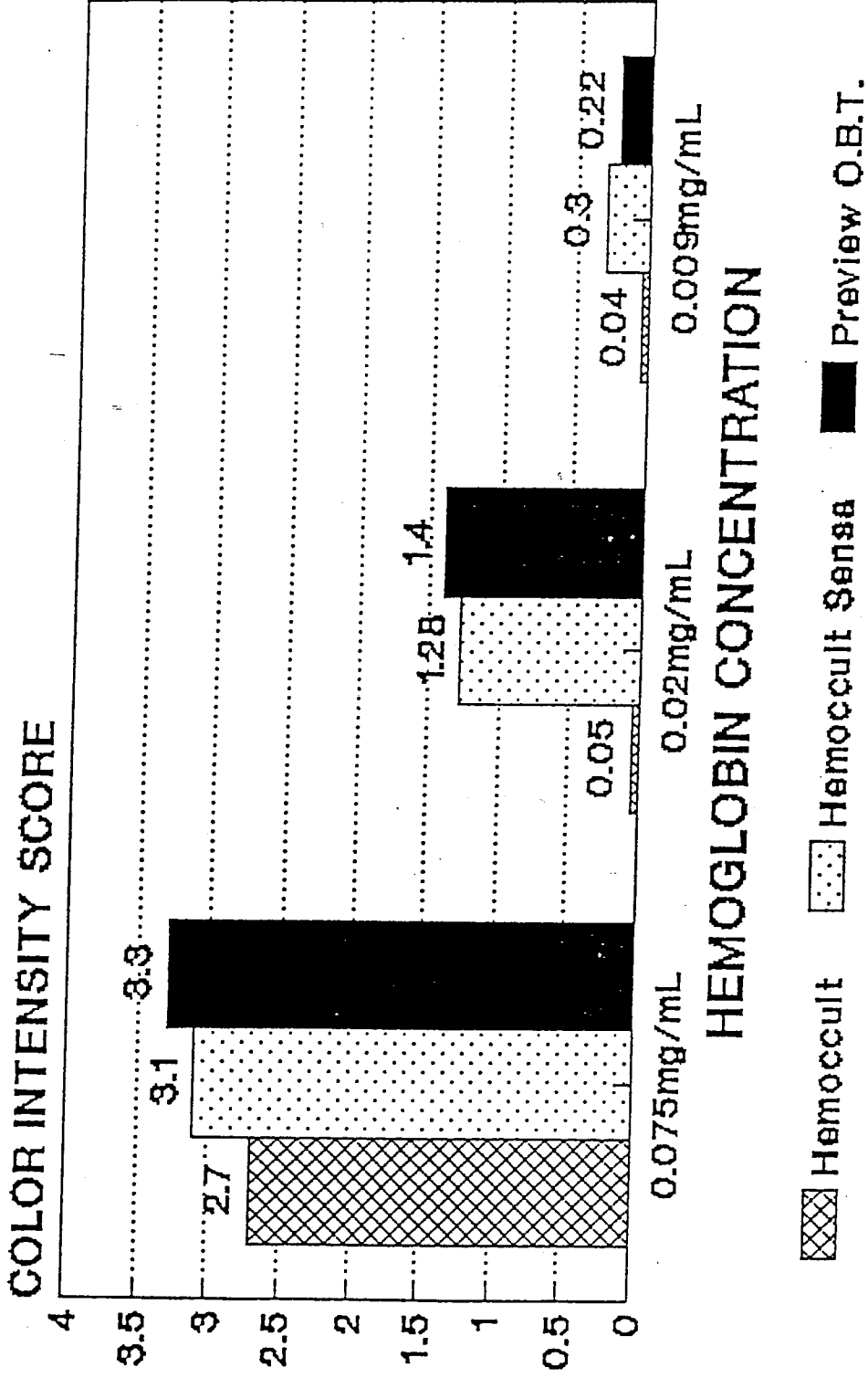
COMPARATIVE DILUTION RESULTS

Read Immediately



COMPARATIVE DILUTION RESULTS

Read At 2 Minutes



Hemocult
 Hemocult Sensa
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