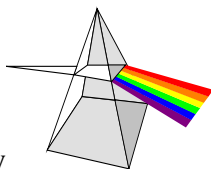


S my



***Frontier Analysis, Ltd.***

**TECHNICAL SERVICE RESPONSE NO.: UT005**

**Subject:** Analysis of Red-Brown Particulates from a Bovine Excision Discovered 1-17-97 (Barton Ranch, Red Bluff, CA)

**Date:** July 19, 2001

**Requested By:** W. C. Levensood  
Pinelandia Biophysical Lab.  
Grass Lake, MI

**Reported By:** P. A. Budinger  
Analytical Scientist

**Objective/Background:**

Identify two samples of red-brown particles retrieved from the testicles and chest area of an excised bull. The bull was found on the Barton Ranch on 1-17-97, around 24 hours after the excision happened. A compass was reported to give a 20° deviation when held over the excisions.

**Conclusions:**

Both residues are identified as bovine hemoglobin, a component of blood. It's presence suggests a processing of the whole blood has occurred at the excision site. Additionally, the chest area sample has a very small amount of possibly a long chain glyceryl ester.

The usual procedure for isolating hemoglobin from whole blood is rather complex. It involves separating red blood corpuscles from the lighter plasma components by centrifugation. The plasma is siphoned off and ether is added to the corpuscle paste, causing the cells to burst. Another centrifugation removes the ruptured cell envelopes, and leaves a clear red solution of hemoglobin.<sup>1</sup> It is unlikely that a procedure such as this would be done on site. It is unknown how or why this occurred.

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<sup>1</sup> Merck Index, 12<sup>th</sup> Ed., S. Budabari, Ed., p. 794, # 4682 (1996).

### **Procedure:**

Sample: There are two samples with the same sample number which are identified as:

- KS-03-181 S1 Red-Brown Material from Bull Testicles
- KS-03-181 S2 Red-Brown Material from Bull Chest Area

The following drawing was submitted with the samples showing their locations.



### **References:**

- Hemoglobin (Hb) Bovine Lyophilized Powder Sigma Cat, No. H-2500
- Whole Bovine Blood, dried at ambient temperature
- Whole Bovine Blood, exposed to microwaves and dried
- Whole Bovine Blood, after 1 week air exposure and dried over that period of time.

Infrared spectra were obtained from both samples as well as references of hemoglobin and whole bloods using the Harrick SplitPea™ cell attached to a Nicolet Avatar 360 spectrometer. The ATR crystal used was silicon. Microscope photographs were obtained from the Leica GZ6 stereomicroscope interfaced to a Kodak Digital Science MDS 120 camera. Additional SEM/EDX elemental information from the bull testicle sample was procured by Dr. Levengood and is included in this report for the purpose of consolidating all the analytical data in one place.

### **Results:**

Microscope pictures of the two samples follow. These were taken at 60X magnification.

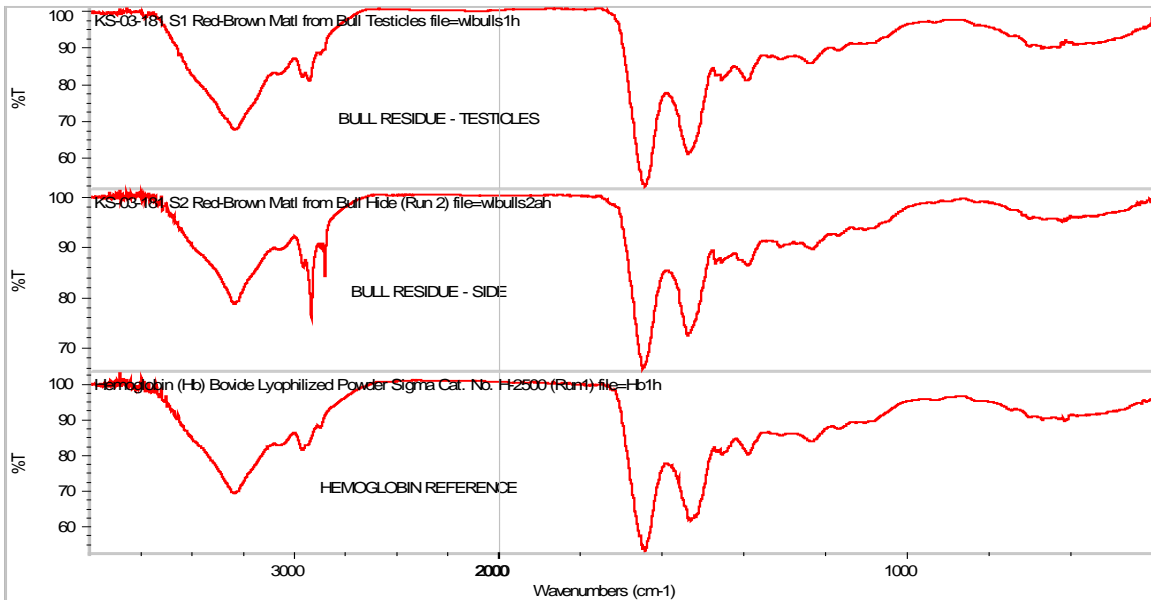


**S1 – Bull Testicle Sample**

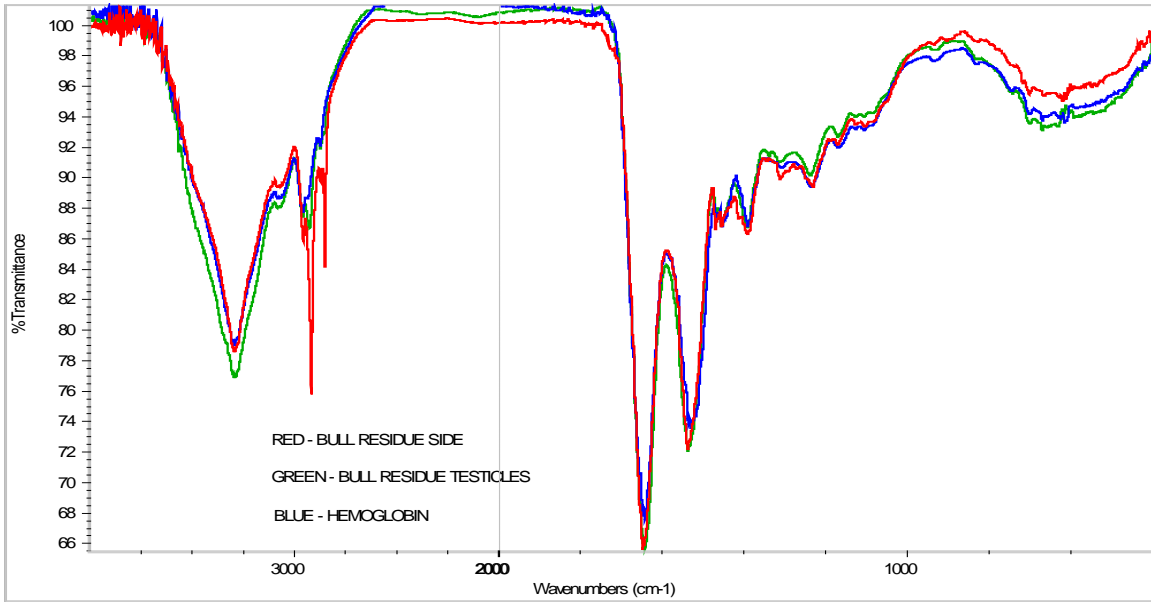


**S2 – Bull Chest Sample**

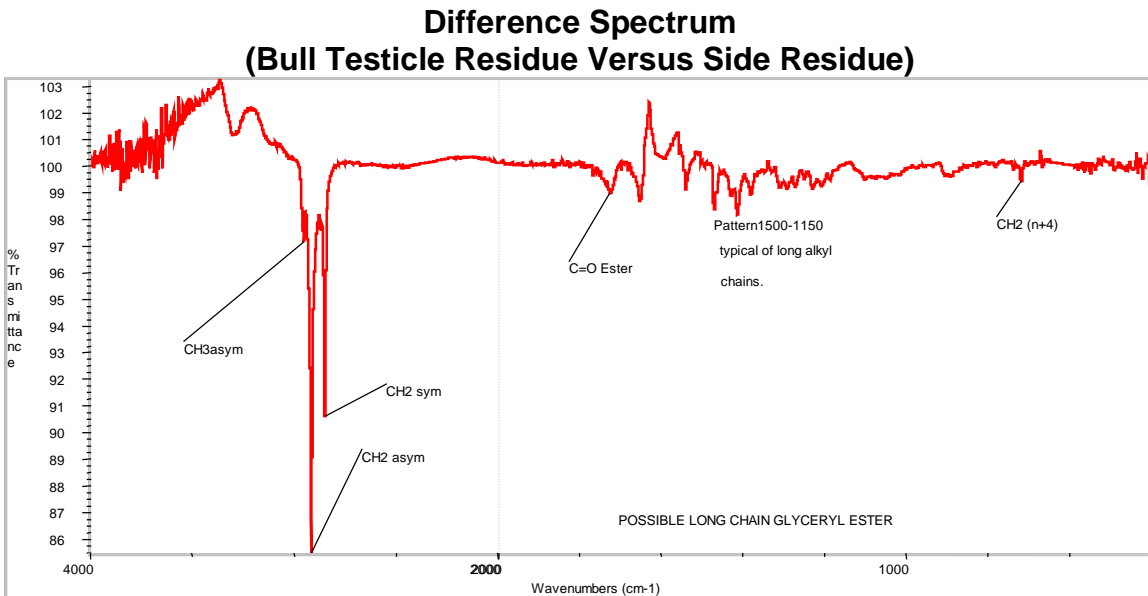
Infrared spectroscopy identifies both residue samples from the excised bull as bovine hemoglobin, a component of blood. Pertinent bands in the infrared spectra of the samples compare to those in a reference of pure bovine hemoglobin purchased from Sigma Aldrich. Additionally, the spectrum of the bull side residue shows absorption in the C-H stretching region between 2800-3000  $\text{cm}^{-1}$ . This absorption plus additional bands are enhanced in a difference spectrum generated between the bull testicle and side spectra. The difference spectrum suggests a long chain glyceryl type ester. Following are the spectra of the two excised bull residues and a reference of hemoglobin for comparison.



The above spectra are superimposed below to more clearly observe the similarities.



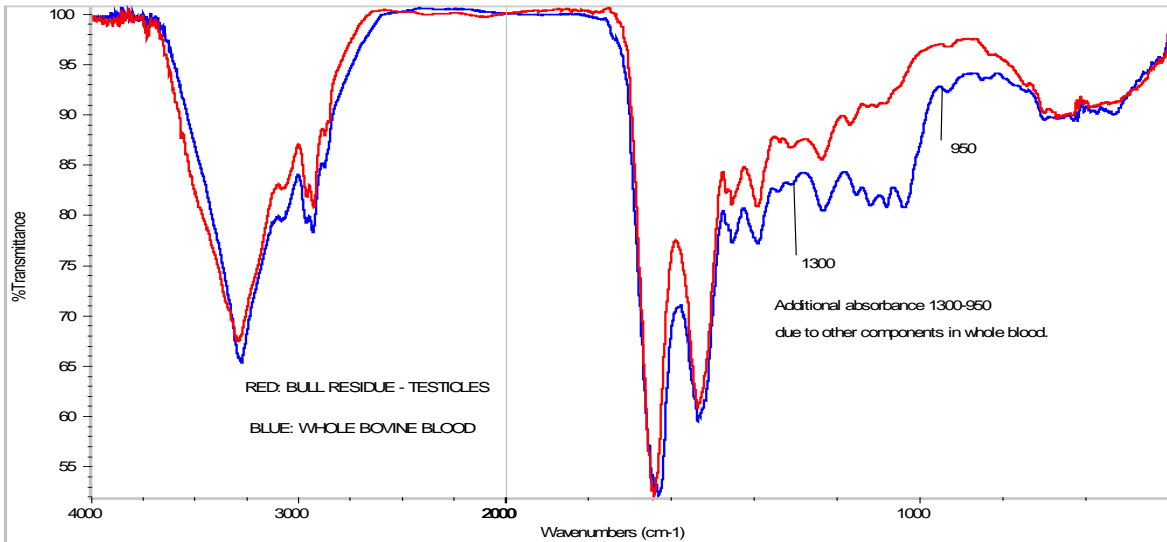
Following is a difference spectrum accentuating the additional component noted in the bull side residue.



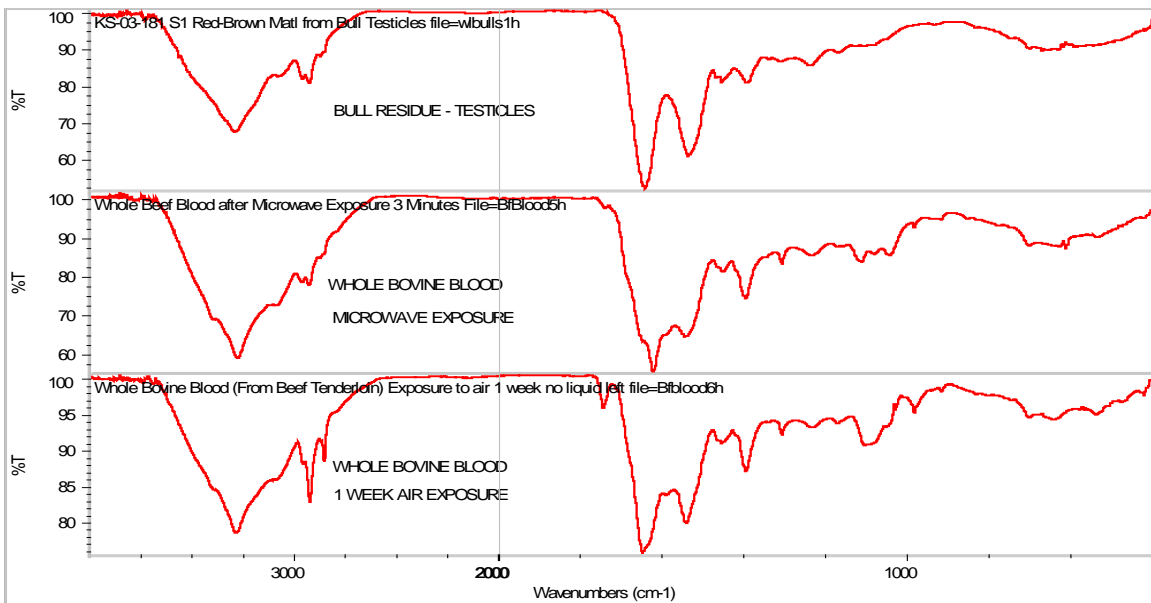
The spectra of the bull residues do not match that of whole blood dried at ambient temperature in the laboratory. The whole blood shows additional absorbance and bands between  $1300\text{--}950\text{ cm}^{-1}$ . The dried whole blood spectrum represents a complex mixture of components such as erythrocytes (red cells), leucocytes (white cells), platelets, plasma proteins and serum. Hemoglobin is a protein found in the erythrocytes<sup>2</sup>. As noted above, the spectra of the bull

<sup>2</sup> The Condensed Chemical Dictionary, 10<sup>th</sup> Ed., Gessner G. Hawley, p.140, (1981).

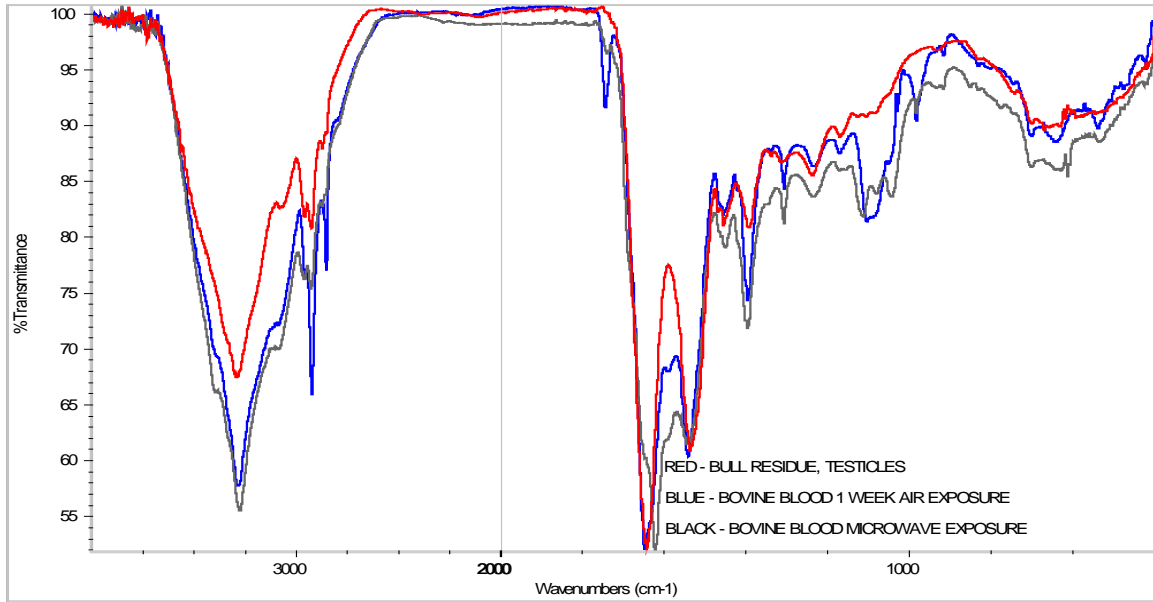
samples specifically show hemoglobin. Spectra of the whole blood and the bull testicle residue are superimposed to show the differences.



Additional spectra were obtained of dried whole blood exposed to microwaves and after one week air exposure. There are significant spectral changes due to chemical alteration that results from microwaves and the air exposure (oxidation). None of these spectra compare to those of the residues retrieved from the bull. Following are the spectra from the whole blood experiments along with a residue from the excised bull for comparison, which clearly shows the differences.



Following are the above spectra which are superimposed to more clearly view the differences.



SEM/EDX analysis shows major elements typical of hemoglobin. These are C, N, O, and Fe. Small amounts of trace elements typical of biological samples are also detected. The detailed information is attached.

File: TSRUT005.DOC

Phyllis A. Budinger

cc.: Nancy Talbott  
BLT Research  
Cambridge, Mass

**SEM/EDX Data (Bull Testicle Sample)**

*KS-03-181*

