Polymorphonuclear (PMN) leukocytes are known to generate light chemiluminescence (CL), emission of light, subsequent to phagocytosis. The CL response is considered to result from relaxation of electronically-excited carbon groups generated during single microbe oxidation of the ingested microbe (Tanai and Qule, 1981). This high energy oxygen molecule in turn is thought to excite other carbon compounds within the PMN (Eason et al., 1980). The phenomenon of CL generated by PMNs was reported originally by Allen et al., (1972) and is associated with increased glucose oxidase via the hexose monophosphate shunt. Reactive oxygen radicals are formed by PMNs during phagocytosis. The sensitivity of CL assay is enhanced by luminol (3-amino-1,2-dihydro-1,4-phenylythiazolone) which reacts with single oxygen and other electronically excited compounds to increase the amount of light emitted. (Kruch et al., 1978; Eason et al., 1980). When both leukocytes and bacteria are kept constant, the rate of CL reflects the ingestion capacity of sera used for opsonization, which may be indicative of prior exposure to that organism. The CL response has been observed to diagnose chronic granulomatous disease (Stjernholm et al., 1973). This assay has also been used to examine the susceptibility of serum to infection (Allen, R.; 1977; Stevens, P. and Young, L.S., 1977). In the present study we examined leukocytes isolated from the blood of swine affected with swine dysentery (SD) or convalescent to the disease to determine if CL could be useful as a diagnostic tool for the disease.

Blood was collected from the cranial vena cana of swine with preservative-free heparin (10 IU/ml). The PMN leukocytes were collected from the same syringe by 5 ml of 6% solution of Dextran (mol. wt. 282,000) in 0.94 saline solution allowed to settle for 1/2 hour at room temperature. Then with the same needle bent 45° the PMNs were transferred to 15 ml sterile polypropylene test tubes and centrifuged at 2000 RPM for 30 minutes. After centrifugation the plasma was discarded and the supernatant was treated with 5 ml buffered NaCl for 5 minutes to lyse and EBC. An equal volume of citrate saline solution was then added to restore lysed cells and the mixture was centrifuged at 2000 RPM for 10 minutes. The cells were then washed 2x in Earl's Balanced Salt Solution (HBSS) and incubated with the Noyel Counter (Am. Scientific Co., Atlanta, GA). Finally, the PMNs were adjusted to a centrifugation of 5 x 10^6 in complete Hank's Balanced Salt Solution (HBSS) with calcium and magnesium and without phenol red the PMNs were 62% of the cell pellet.

Luminol was converted to a soluble potassium salt by dissolving 0.012 g luminol, 0.78 g potassium hydroxide and 0.018 g boric acid in 10 ml distilled water. This stock solution was wrapped in aluminum and allowed to settle for three days at 4°C. Two hours prior to use, an aliquot of stock solution was diluted to 1:80 in sterile water and kept at 4°C. This solution was used in all experiments. Luminol hypochromic toner was grown in broth culture as previously described (Jenkins et al., 1979).

The reaction mixture (a 1:1 ratio of luminol to PMNs) consisted of 0.1 ml PMN suspension in complete, cold HBSS, 0.05 ml luminol diluted in complete HBSS and 0.1 ml suspension in 6x 50 mm glass reaction tubes. The final volume in the reaction tube remained constant throughout all experiments. The mixture was vortexed briefly and placed in the light-proof chamber of the Pico-Lite Luminometer from Packard Instruments (Downers Grove, IL). All constituents were kept at room temperature prior to assay, and the assay was run at 37°C. Counts were printed at 30 second intervals until peak emission was recorded. The CL responses were run in triplicate and the mean recorded as Counts Per Minutes (CPM).

The results of the experiments indicate that fresh PMNs (1-8 hrs) gave a significantly greater (P < 0.05) CL response than older cell (1-8 hrs). Likewise, there was no significant difference (P > 0.05) in the CL response of cells obtained from normal animals, those of SD-negative pigs and those of infected ones. A 1:20 dilution of serum consistently gave the maximum CL response which was determined by titrating with hyperimmune anti-T. hydysenteriae serum. Heat inactivated sera from 80% infected swine resulted in a reduction of activity indicating opsonin-dependent CL. The mean and standard error of the mean CPM of 3 T. hydysenteriae infected pigs were significantly lower (P < 0.05) as determined by the Student's "t" test than that of a comparable number of normal pigs (2.110*4.58*106 vs 6.565*2.12*106, respectively). The CL response of the serum against antigens of Salmonella cheifensis (SC) and enteropathogenic Escherichia coli (EPEC) resulted in one half to one third of the response of those of T. hydysenteriae. However, when sera obtained before and after infection with T. hydysenteriae were absorbed with SC and EPEC there was a significant reduction (P < 0.05) in CL response in both, indicating lack of specificity of the assay. Our results indicated that a significantly greater difference in the CL response of the sera of T. hydysenteriae infected compared to those of normal or uninfected pigs. However, absorption of these sera with heterologous organisms significantly reduced the CL response specific to T. hydysenteriae. Therefore, the usefulness of this test as a diagnostic trial for field cases of SD is questionable.