

Spring 5-2015

## **A Comparison of Cyfra 21-1, NSE, and CEA For the Serodiagnosis of Lung Cancer**

A Alexandra Knopp  
*University of Southern Mississippi*

Follow this and additional works at: [https://aquila.usm.edu/honors\\_theses](https://aquila.usm.edu/honors_theses)



Part of the [Diagnosis Commons](#)

---

### **Recommended Citation**

Knopp, A Alexandra, "A Comparison of Cyfra 21-1, NSE, and CEA For the Serodiagnosis of Lung Cancer" (2015). *Honors Theses*. 301.

[https://aquila.usm.edu/honors\\_theses/301](https://aquila.usm.edu/honors_theses/301)

This Honors College Thesis is brought to you for free and open access by the Honors College at The Aquila Digital Community. It has been accepted for inclusion in Honors Theses by an authorized administrator of The Aquila Digital Community. For more information, please contact [Joshua.Cromwell@usm.edu](mailto:Joshua.Cromwell@usm.edu), [Jennie.Vance@usm.edu](mailto:Jennie.Vance@usm.edu).

The University of Southern Mississippi

A COMPARISON OF CYFRA 21-1, NSE, AND CEA FOR THE SERODIAGNOSIS  
OF LUNG CANCER

by

Alexandra Knopp

A Thesis  
Submitted to the Honors College  
of the University of Southern Mississippi  
in Partial Fulfillment  
of the Requirements for the Degree of  
Bachelor of Science  
in the Department of Chemistry and Biochemistry

May 2015



Approved by

---

Margot Hall, Ph.D., Thesis Adviser  
Professor of Medical Laboratory Science

---

Mary Lux, Ph.D., Chair  
Department of Medical Laboratory Science

---

Ellen Weinauer, Ph.D., Dean  
Honors College

Graduated with a degree in Chemistry (Biochemistry)

## Abstract

There were 224,210 new cases of lung cancer in the US during 2014, and of those cases, there 159,260 that died from lung cancer during 2014. Since early diagnosis and treatment leads to a better prognosis, the medical community is actively looking for new, noninvasive tests for the disease. This includes the search for new and effective tumor markers. Tumor markers are used in combination with other tests to diagnose cancer. After the diagnosis, they are used to follow a patient's case. The three tumor markers studied were neuron specific enolase (NSE), carcinoembryonic antigen (CEA), and CYFRA 21-1. In this study, the normal reference intervals were developed using sera from healthy donors. The analytical properties of the tumor marker assays were tested for and found to be satisfactory. The study was designed to compare the diagnostic and predictive values for the three tumor markers. Preliminary results on 638 patients (76 lung cancer patients, 562 healthy patients) included: (1) diagnostic % sensitivity (CEA 22.37%, NSE 0%, CYFRA 21-1 18.92%), (2) diagnostic % specificity (CEA 80.43%, NSE 99.39%, CYFRA 21-1 93.16%), (3) %PV+ (CEA 13.39%, NSE 4.17%, CYFRA 21-1 28.00%), (4) %PV- (CEA 88.45%, NSE 87.12%, CYFRA 21-1 89.09%), (5) %efficiency (CEA 73.51, NSE 86.65%, CYFRA 21-1 84.00%). It was hypothesized that CYFRA 21-1 would be superior to CEA and NSE for the sero-diagnosis of lung cancer in a cohort of patients, and the hypothesis was rejected. CYFRA 21-1 was not superior to CEA and NSE in the sero-diagnosis of lung cancer.

**Key Terms:** Cancer, Carcinembryonic Antigen, Neuron Specific Enolase, CYFRA 21-1,  
Lung Cancer

## **Acknowledgements**

First of all, I would like to thank my parents because, without them, I would not have been able to go to school to achieve my dreams and get as far as I have today. I would like to thank them for all of their love and endless support. Second of all, I would like to thank Dr. Hall because without her help and expertise and hours of time spent I would never have been able to complete the lab work and put this manuscript together. She has been an amazing mentor and such a positive influence on my life throughout my college career. I will cherish all of the knowledge and experiences I have gained from this project with her.

## Table of Contents

Introduction .....	1
Literature Review.....	2
Materials and Methods.....	21
Results.....	29
Discussion.....	37
Conclusion .....	38
References.....	39
Appendices.....	43

## Table of Graphs

Graph 1: CEA Linearity Plot .....	34
Graph 2: CYFRA 21-1 Linearity Plot.....	35
Graph 3: NSE Linearity Plot.....	36



## List of Tables

Table 1: Patient Sample Classification .....	22
Table 2: Specificity and Sensitivity per % concentration.....	28
Table 3: Within Run Assay Precision for NSE, CYFRA 21-1, and CEA .....	31
Table 4: Between Run Precision for NSE, CYFRA 21-1, and CEA .....	31
Table 5: Assay Linearity for NSE, CYFRA 21-1, and CEA .....	32
Table 6: Analytical Sensitivity for NSE, CYFRA 21-1, and CEA.....	32
Table 7: Normal Reference Intervals for NSE, CYFRA 21-1, and CEA .....	33
Table 8: Predictive values for NSE, CYFRA 21-1, and CEA in 638 Patients .....	33

## **Introduction**

### **Epidemiology**

In the US, there were 1,665,540 cases of all types of cancer in 2014 and 585,720 resulting deaths in 2014 (American Cancer Society, 2014). Since early diagnosis and treatment leads to a better prognosis, the medical community is actively looking for new noninvasive tests for the disease. This includes the search for new and effective tumor markers.

### **Objective of Study**

The objective of this study was to compare and evaluate three tumor markers, CYFRA 21-1, carcino embryonic antigen (CEA), and neuron specific enolase (NSE) for the sero-diagnosis of lung cancer.

The tumor marker CYFRA 21-1 is used to diagnose lung cancer, but it has also proved successful in identifying other tumors. It can be a marker for cancers of the head and neck. It also has proved successful in monitoring tumors in the cervix and has been considered useful in identifying non-small cell lung cancer (NSCLC). This includes squamous cell carcinoma (SCC), adenocarcinoma, and large cell carcinoma. These types of tumors account for 80% of the lung tumors (Nakamura & Wu, 1997).

CEA is a marker that has been used for colorectal cancer, renal cancer, ovarian cancer, and breast cancer. It was first discovered in extracts of colon cancer. It was thought that a tumor specific marker had been found, but it was later discovered that not all colon tumors produced CEA. This is because tumors are very heterogeneous in their composition. Similarly, elevated blood CEA has been observed in heavy smokers who were tumor free. It is used as a minor marker in lung cancer (Nakamura & Wu, 1997).

NSE is a soluble metal-activated glycolytic metalloenzyme that provides components necessary for aerobic glycolysis. Decreasing values of this enzyme after primary treatment corresponding to the half-life period is the first sign of a good prognosis and good treatment effect. NSE can play no role in the staging of the disease. It was also found unable to differentiate between partial and complete response to treatment (Schneider et al., 2002).

### **Hypothesis**

It is hypothesized that CYFRA 21-1 will be superior to CEA and NSE for the sero- diagnosis of lung cancer in a cohort of patients.

## **Literature Review**

### **Cancer**

**History.** During the past 150 years, infectious diseases have been replaced by arterial disease and cancer as the major causes of death. Today, arterial disease accounts for 50% of all deaths in the US, and cancer accounts for 20% of deaths in the US. Heart attacks and strokes, which are associated with arterial disease, are seen as hazards of old age, lack of exercise, and poor diet. Cancer, though, is thought of as an unpredictable disease. It strikes no matter how old or how fit one is. This seems to be true because cancer can be related to environmental factors (Conklin, 1949).

**Nomenclature.** A carcinogen is a substance that causes cancer. Some chemical carcinogens can act directly, but others require metabolism in vivo before becoming effective. Viruses are submicroscopic particles that contain either DNA or RNA. Both DNA and RNA are long strands of four of the chemical units called nucleotides. Tumor viruses can elicit cancerous changes in cells. Oncogenes are genes whose protein

products may be involved in processes leading to transformation of a normal cell to a malignant state. The gene may be known as a viral oncogene if it was detected in a transforming virus. A tumor suppressor gene is a gene whose mutation or loss may lead to cellular transformation and to the development of cancer. Angiogenesis is the formation of new blood vessels. This process is essential for tumor growth and appears to be stimulated by endothelial cell growth factors. Metastasis is the spread of cells from a primary tumor to a noncontiguous site, usually via the bloodstream or lymphatics, and the establishment of a secondary growth. Apoptosis is a process resulting in cell death due to the activation of a genetic program that causes cells to lose viability before they lose membrane integrity. The process involves endonuclease mediated cleavage of the DNA into fragments of specific lengths leading to a "DNA ladder" when it is subjected to gel electrophoresis. Apoptosis is also called programmed cell death and is important in maintaining tissue homeostasis; it may be important in the response of tumor and normal tissue cells to therapeutic agents (Bristow, Harrington, Hill, & Tannock, 2005)

**Epidemiology.** There were 1,638,910 new cases of cancer in the US in 2012. Also, in 2012, there were 577,190 deaths in the US due to cancer. In 2005, seven years earlier, the statistics for new cases and deaths from those cases were close to the same. The number of new cases was 1,372,910, and the number of deaths out of those cases was 570,280. Despite medical advances, the prevalence and incidence has not improved. That is why there is such an extreme importance for studying cancer. (American Cancer Society)

**Major types of cancer.** Some major types of cancer are lung, breast, colorectal, stomach, prostate, cervical, ovarian, testicular and bladder. The tumor markers Cyfra 21-

1 and CEA identify two or more of these major types of cancer. Cyfra 21-1 has been reported to be elevated in patients with tumors of the lungs and in cervical cancer. Elevated CEA has been reported with lung, breast, colorectal, stomach, prostate, and bladder tumors. CEA is a protein found on the surface of the above listed cancers that it identifies with and some embryonic cell types. These tumors are of epithelial cell origin and were derived from endoderm during embryonic development. Thus, they share some common properties (Cooper, 1992). The tumor marker NSE is associated with neuroendocrine tumors. There will be heightened NSE antigen with carcinoid tumors, endocrine pancreatic tumors, pheochromocytoma, medullary thyroid cancer, and neuroblastoma (Schneider et al., 2002).

**Lifestyle choices.** The incidence of lung cancer in western countries is directly proportional to the amount of cigarettes its inhabitants smoked 10 to 20 years earlier. The amount of cigarettes smoked in the western countries is completely irrelevant to incidence of lung cancer during that time period. The damage has to have been done to the body years earlier than when the lung cancer occurs (Cairns, 1975).

Just as the choice to smoke cigarettes influences the chance of someone developing lung cancer years later, a person's occupational choice can have the same effect. Occupational cancers are those that are due to exposure to industrial chemicals (e.g. benzene) while working. These cancers may not appear until 10 to 20 years after the person has retired (Cairns, 1975).

Another lifestyle choice that can have an effect is the amount of exposure to the sun. Sunbathing can lead to skin cancer. People with fair skin are more likely to develop skin cancer than those with darker skin. Another occupational hazard involves working

with x-ray machines and the need to wear lead aprons. Also, they have started shooting the x-rays from a distance. Before these safety measures were instituted, there was more prevalence of cancer among x-ray technicians.

**Chemical carcinogens.** Chemical carcinogens can cause cancer. Some chemicals are electrophiles, meaning that they are looking for an electron to become more stable. This is the reason that they will bind to the first contacted electron donor. Carcinogens could be in a nose cell, a lung cell, or a skin cell, where they bind to the DNA. The polymerase then starts trying to copy the DNA but the chemical carcinogen is in the way. The polymerase may skip that spot or move to the other side and start copying there. Some organic chemicals, which are carcinogens, include anthracene oil, aromatic rings, Benzol, Carbon black, Creosote, mineral oils, paraffin oil, polycyclic hydrocarbons, shale oil, soot, tar and pitch, aniline, Azo dyes, and Estrogens (Conklin, 1949).

**Viruses.** Viruses are packets of genetic information encased in a protein. The information can be embodied in either DNA or RNA (Bishop, 1982). The central dogma of molecular biology held that information sequentially transferred from DNA to RNA to protein. There are some viruses that have a reverse flow of information that goes from RNA to DNA. This group of viruses that has a reverse flow of information also provides a reverse transcriptase enzyme to synthesize cDNA (complementary DNA) from RNA. Many of these viruses produce cancer in animals and have also been linked to transformations in humans. These viruses are known as retroviruses. Retroviruses have been found to infect animals such as mice, birds, minks, cats, and cows. They also infect humans. An example of a retrovirus that infects humans is HIV. HIV has been known to cause tumorigenesis (Richardson, 2005). This concept led to an experiment done in the

1950's with the rous sarcoma virus. A tissue culture assay for the rous sarcoma virus was developed. The assay involved adding suspensions of the virus to sparse cultures of cells taken from the body wall of chicken embryos. The virus infected some cells and turned them into tumor cells. The transformed cells differed in morphology and in growth properties (Temin, 1972).

The number of transformed cells was proportional to the number of infectious units of the virus added to the cell culture. The rous sarcoma virus was different from previous viruses studied in that it did not cause the cell that it infected to die. The cells that were infected with this virus continued to survive and produced virus particles when they divided (Temin, 1972). An infecting virus insinuates its genetic information into the cellular machinery so that the cell synthesizes viral proteins specified by viral genes (Bishop, 1982).

**Oncogenes and tumor suppressor genes.** Although genes implicated in the development of cancer were first observed to work with in viruses, they are not only found in viruses. The genes are not limited to being a part of cancer cells. They are present, functioning, and necessary for the life of the normal cell. Oncogenes serve as the code for proteins which contribute to cellular proliferation that is necessary for growth and development as well as wound healing (Bishop, 1982).

An oncogene is a gene whose protein product may be involved in processes leading to transformation of a normal cell to a malignant state. Oncogenes are the genes that cause cancer. They are altered versions of ordinarily benign genes present in normal cells. A normal oncogene encodes proteins. Proteins are the substances that provide the structure of the living cell and catalyze its biochemistry. The proteins are what determine

a cell's form and function. Once the oncogene is transformed, the proteins encoded by it function abnormally and transform a normal cell into a cancer cell (Hunter, 1984).

A tumor suppressor gene is a gene whose mutation or loss may lead to cellular transformation and to the development of cancer. Oncogenes are dominant, and tumor suppressor genes are recessive. They are also known as recessive oncogenes or anti-oncogenes. Their inactivation can lead to a loss of function mutation. Oncogene activation and tumor suppressor gene inactivation allow a stepwise progression of tumorigenesis (Oster, Penn, & Stambolic, 2005).

**Growth Factors, Receptors, and Signaling.** In multicellular organisms, cell regulation is controlled by polypeptide molecules called growth factors. These growth factors interact with receptors on the cell surface, which leads to the modification of intracellular biochemical signaling pathways that control cellular responses such as cell proliferation. Receptors for growth factors are molecules that span the membrane of the cell surface and have the ability to phosphorylate themselves and other cytoplasmic proteins on tyrosine residues activating a cascade of signals. These receptor molecules are called receptor protein tyrosine kinases. When the growth factor binds, it induces conformational changes in the extracellular domain of the receptor that facilitates dimerization. The dimerization results in phosphophorylation. There are some abnormal receptor proteins. Their abnormality can be caused by denaturation, which is when a protein is damaged by heat, and the area where the growth factor is supposed to bind is not the right shape and size. This leads to the growth factor either not binding at all or the wrong growth factor binding to the site. This produces continuous and inappropriate cellular signaling, which could lead to increased cellular proliferation. This occurs



because the signaling pathways that the receptor proteins are stimulating control cellular proliferation (McGill & McGlade, 2005).

**MicroRNAs.** MicroRNAs are short non-coding RNAs of 20-24 nucleotides that play important roles in virtually all-biological pathways in mammals. MiRNAs influence numerous cancer relevant processes such as proliferation, cell cycle control, apoptosis, differentiation, migration, and metabolism. A single miRNA may target up to several hundred mRNAs and affect multiple transcripts, which influence cancer related signaling pathways. MiRNAs have a tight integration in the cellular regulatory circuits. This can be the downfall for a cell. The deregulation of a small subset of miRNAs may affect the expression pattern and drive the cells toward transformation. MiRNAs are generally down-regulated in cancer, and tumors often present reduced levels of mature miRNAs. This is because the tumors have genetic loss, epigenetic silencing, defects in their biogenesis pathways, and transcriptional regression. Because of miRNAs involvement in the regulation of the cellular processes, they are a promising therapeutic tool, even though the study has a long way to go for the full understanding of the mechanisms (Jansson & Lund, 2012).

**Radiation carcinogenesis.** Humans are exposed to low levels of radiation throughout life. These low levels of radiation have been studied and no real harm comes from them usually. However there are certain types of radiation that have a cumulative effect. Damage to the DNA is the most critical effect of low-level radiation. Damage to the chromosomes is often repaired by the body, but it is sometimes misrepaired. This results in chromosomal abnormalities. These chromosomal abnormalities can lead to cancer because the gene now is not functioning correctly. There have been people that

have had cancer caused by radiation therapy. The radiation is supposed to pass through the tissues to get to the tumor to kill it and make it stop proliferating, but while doing that, it damages the tissues around it and the tissues it passes through (Upton, 1982).

Other forms of radiation that can be harmful to some people are those that they are involved with in their occupation. There are cancer cases among radium-dial painters, radiologists, uranium and other miners, and nuclear workers. They are exposed to radiation over time, and it has a cumulative effect, which can cause cancer (Upton, 1982).

**Cell proliferation and tumor growth.** Normal cell cycle progression is a highly regulated series of events. There are two phases in the cell cycle called the S phase (DNA Synthesis) and the M phase (mitosis). The gap between the M and S phase is called G1. The gap between the S phase and the M phase is called G2. After the M phase, cells may enter the G0 phase if there is no stimulus to further divide. Once there is a stimulus, cells in the G0 or G1 progress to a restriction point R. In cancer cells, there is deregulation of control mechanisms such that when the cell receives a stimulus it is more likely to proceed through check point R. That is how tumor growth begins. During tumor development, the cell cycle is constantly deregulated. The progression of the cancer, which allows for the continuing development of the tumor, involves processes that abort differentiation, allow immortalization, and promote loss of sensitivity to growth inhibiting stimuli (Donovan, Slingerland, & Tannock, 2005).

Differentiation is the development of cells that have specific morphological and bio-chemical characteristics that control and describe all the normal functions of a particular tissue. In cancer cells, the processes that promote differentiation are aborted, so the cells that develop the tumor are different from the normal tissues cells around them.

They do not have the characteristics that promote normal function (Donovan et al., 2005).

Normal cells do not proliferate indefinitely. They have a proliferative potential that is constrained to a definite number of population doublings. This leads to a terminal growth arrest or senescence. Senescence functions as a form of tumor suppression because it will stop proliferation, limiting genetic errors in a cell. When a cell loses its ability to stop proliferating, it becomes immortal and has uncontrolled cell proliferation (Donovan et al., 2005).

Once the cells are proliferating uncontrollably, they have also lost the ability to be sensitive to growth inhibiting stimuli. This means that when there are too many cells in an area, they will not stop proliferating. The cancer cells begin to form clumps and stack on top of each as they proliferate. That is how a tumor forms (Donovan et al., 2005).

**Tumor progression and metastasis.** Most patients do not die from a single tumor. They ultimately die because of the progression of the tumor and its metastasis. Metastasis is the spread of multiple tumor colonies that have detached themselves from the original tumor and moved via blood and lymph to other tissues in other parts of the body (Nicolson, 1979).

Studies have shown that cells from certain cancer types (tumors) will only travel to certain other tissues and areas of the body. Breast carcinomas tend to spread to the brain or lung. A study was done where the cells were injected above (upstream) and below (downstream) the area of study. The cells would find the area they were specific to even if they had to travel around the body to get back to it after their point of injection. Once the cancer cells do reach the blood stream their chance of survival decreases. The

fast movement of the bloodstream will kill some of the individual cancer cells. A study was done in which melanoma cells were injected into the bloodstream of mice. Within a few minutes most of the melanoma cells had died. It is very hard for the cells to survive the fast movement and hostile environment of the blood stream (Nicolson, 1979).

**Angiogenesis.** Angiogenesis is the critical event that converts a self-contained packet of cells into a group of malignant cells. The malignancy happens when the tumor becomes vascularized. At this point the tumor has induced the host to provide it with its own network of blood vessels. The tumor induces the host to provide these blood vessels by releasing a chemical substance named tumor angiogenesis factor or TAF. The TAF stimulates the nearby blood vessels to send out new capillaries that will grow toward the tumor and eventually penetrate it. Once the tumor is vascularized it can bring in its own nutrients through the vessels and deplete its waste as well. This is why rapid growth follows (Folkman, 1976).

**Cell death (apoptosis).** Apoptosis is a process resulting in cell death due to the activation of a genetic program that causes cells to lose viability before they lose membrane integrity. Apoptosis is also called programmed cell death and is important in maintaining tissue homeostasis. It may be important in the response of tumor and normal tissue cells to therapeutic agents directed against cancer. Apoptosis is a genetically regulated response. It can be triggered by growth factor deprivation or other DNA damaging agents like radiation or chemotherapeutic drugs (Hakem & Harrington, 2005).

### **Cancer treatment.**

***Hormone therapy used in prostate cancer.*** There is now hormone therapy to slow the growth of the hormone dependent tumors, such as in the prostate. The LHRH

(Luteinizing Hormone Releasing Hormone) is normally released from the hypothalamus in pulses. This leads to the pulsatile release of FSH (Follicle Stimulating Hormone) and LH (Luteinizing Hormone). The LH attaches to the receptors on the Leydig cells of the testes. This promotes testosterone production. Testosterone can be converted into dihydrotestosterone. Testosterone and dihydrotestosterone will feed back in a negative feed back loop and turn off the gonadotropin releasing hormone so that no more LH will to stimulate the release of testosterone (Hellerstedt & Pienta, 2002).

There are methods of primary androgen ablation that helps eliminate prostate cancer. Sometimes an agonist for LHRH is used. This will stimulate the release of LHRH. Then testosterone will be produced and all of the androgenic receptors will be occupied where no more testosterone is produced. This can be a problem because there is an initial surge of testosterone. Cyproterone acetate is another treatment involved with the hormones that make testosterone. It is a steroidal progestational antiandrogen that blocks the androgen receptor interaction and reduces serum testosterone (Hellerstedt & Pienta, 2002). The reduction of the testosterone will help slow the growth of the prostate cancer.

***Monoclonal antibodies.*** The antigens on the surface of the tumor cells are studied. The tumor antigens that are expressed are selected for antibody targeting. Therapy with antibodies requires a comprehensive analysis of tumor expression and normal tissue expression so that there is understanding of the biological role of the antigen in the tumor growth. There are several mechanisms of tumor cell killing by antibodies. This is why the antibodies and antigens have to form a particular complex, and this complex cannot be internalized by the cell unless a drug is being delivered to the

tumor cell. These mechanisms of tumor cell killing by antibodies are direct cell killing mechanisms through receptor blockade or agonist activity, induction of apoptosis, or delivery of a drug, radiation, or cytotoxic agent. This is when the cell internalizes the antibody-antigen complex. The mechanisms of tumor cell killing can also be through immune-mediated cell killing mechanisms and regulation of T cell function and specific effects on tumor vasculature and stroma (Allison, Scott, & Wolchok, 2012).

***Gamma knife surgery.*** Gamma knife stereotactic radiosurgery was developed as a noninvasive alternative to conventional neurosurgery. It is characterized by a single, high-dose external radiation in mechanically fixed conditions. This system was used to maximize the target dose exposure while minimizing undue exposure of surrounding tissues. This technique was originally used on benign diseases an extraaxial intracranial lesions (Gerosa, 2003).

## **Lung Cancer**

**Epidemiology.** There were 224,210 new cases of lung cancer in the US during 2014, and there were 159,260 of these patients that died during 2014 (American Cancer Society, 2014).

### **Common believed causes of lung cancer.**

***Asbestos.*** Asbestos is a commonly believed cause of cancer. This is not a complete truth. Asbestos cannot cause cancer by itself. It has to have a contributing factor like lung fibrosis. There were 839 men that were studied that were involved in the manufacturing of asbestos containing cement. The study concluded that the men that had lung fibrosis before the start of working there had an increased risk for having asbestos

caused lung cancer. The men that did not have lung fibrosis were not at an increased risk for lung cancer from working with asbestos. Lung fibrosis is caused by smoking or a viral or bacterial infection like pneumonia that leaves dark spots on the lungs (Hughes & Well, 1991).

**Radon.** Radon is another commonly believed cause of cancer. It is one of the most extensively studied human lung carcinogens. Underground miners and their occupational study is where most of the evidence is derived from. Underground miners are exposed to much higher radon levels than are what are commonly found in the household. It is clear that underground mine workers have an excess risk for lung cancer. There are much lower doses of radon in the household which makes household exposures being a risk for lung cancer much more unclear (Alavanja et al., 2004).

There are also major differences in the home exposures and the underground exposures that occur in mines. These differences include the relative proportion of radon itself to its decay products, respiratory rate, and particle size distribution. The decay products affect the amount of energy deposited in the lung. The respiratory rate affects the rate radon and its decay products are inhaled and retained in the lung. The particle size distribution affects the fraction of radon progeny attached to particles and the depth of penetration and site of deposition within the lung. This is why it is hard to compare household radon hazard studies to occupational hazard studies of underground mine workers (Alavanja et al., 2004).

It has been found that there is as much cellular DNA damage due to a low level dose over an extended period of time as to a high dose of radon that the underground mine workers receive. It can be concluded from this evidence that residential radon does

account for some lung cancer and that 10-15% of the lung cancer cases are due to residential radon (Alavanha et al., 2004).

***Chromosome breakage.*** In the past ten years, the researchers in genome stability have observed many types of cancers that are associated with chromosome breakage. Breast cancer has been particularly studied with chromosomal breakage (Przybytkowski et al., 2014). When chromosomes break, they normally form back together unchanged and replication continues, and then, the cell divides. But, there are times where the break leads to deletions or rearrangements. The genes being replicated that are in the area where the DNA broke are now changed. Cancer cells generally have some sort of deletion or rearrangement. This is why chromosome breakage is thought to have a medical consequence. What if the cell, during chromosome breakage, loses part of the gene that is needed for the cellular DNA to continue replicating properly in the break correction? What if there was a mutation in the tumor suppressor gene during the correction of the chromosome breakage? Now a rapidly dividing cell has occurred that eventually will form a tumor (Tufts University, 2007).

### **Diagnosis and staging.**

***Computed tomography.*** Computed tomography scanning is based on the measurement of the amount of x-ray weakening as x-rays pass through different tissues within the body. Bone and tissues interact differently with the tomography, producing different attenuation coefficients. Attenuation coefficients are a quantity that characterizes how easily a material or medium can be penetrated by a beam of light. Attenuation coefficients can be calculated as a function of the space in the cross sectional area where the x-rays pass. These different functions of space show up on the two-



dimensional image as different shades of grey in an area. This creates a two-dimensional image and is generally used for chest x-rays and mammograms. If there is a tumor in the lung or in breast tissue, there will be a different attenuation coefficient as compared to that seen with normal lung and breast tissue. CT scanning is also a type of computed tomography. CT scanning is a cross sectional image obtained by exposure to a thin beam of x-rays throughout a 360 degree rotation. Both x-ray imaging and CT scanning provide exclusively anatomical information (Sherar, 2005).

***Nuclear Medicine and Bone Scans.*** Nuclear medicine uses radioactive agents to obtain images of tumors in the patient for diagnosis. The radioactive agents are radioactive isotopes. The radioactive isotopes used for diagnostic imaging emit high-energy photons. The photons are detected by a large sodium iodide crystal scanner, which transfers the photons into light signals. The light signals are then detected using a photomultiplier tube. This type of imaging is used commonly for detecting the presence of metastatic disease to the bone (Sherar, 2005).

***Magnetic resonance imaging.*** Magnetic resonance imaging is based on magnetization of tissues when a patient is placed in a large, externally applied magnetic field contained in a MRI scanner. MRIs have become a commonly used technique for the diagnosis of cancer. MRIs have an excellent soft tissue contrast and resolution. It is excellent for imaging the brain, head, neck, and pelvic region (Sherar, 2005).

***Ultrasound.*** The standard B mode ultrasound is used in diagnosis. The imaging projected from this ultrasound is based on the reflection of very high frequency sound signals. The ultrasound uses a piezoelectric crystal that generates a short ultrasound pulse that penetrates the tissue and is reflected by structures with different mechanical

properties. The image the ultrasound forms is produced by time-gating the signals scattered back to the transducer. The scattering of ultrasound is different between normal tissues and tumors. An ultrasound is particularly useful for diagnosis in the abdomen. However, it will not pass through bone well enough for it to provide proper imaging (Sherar, 2005).

### **Treatments.**

*Surgery.* Surgery can be curative when performed early on in the course of the disease. That is if the tumor is operable (Nakamura & Wu, 1997).

*Chemotherapy.* Patients are treated commonly with chemotherapy. Many cancer drugs are also carcinogens, and all cancer drugs are toxic to normal tissues. They cause toxic damage through effects on DNA and DNA synthesis. Patients who are long-term survivors of chemotherapy are at risk for a second malignant tumor because their DNA and tissues have been damaged. Many secondary malignancies are acute leukemias. Toxicity to the normal tissues limits both the dose and the frequency of drug administration (Boyer & Tannock, 2005).

*Methotrexate as a form of chemotherapy.* Methotrexate is a powerful, versatile drug that is used in cancer chemotherapy. It is a folic acid antagonist, so it inhibits DNA synthesis. It is used both orally and intra-arterial infusion (Boulter, 1967). There are acute manifestations of damage seen in multiple organs after doses of methotrexate. The acute manifestations are first seen in the gastrointestinal tract. It will cause the suppression of the gut epithelial cell mitosis in the crypts. This will eventually lead to ulcers. These ulcers have also been seen in the mucous membranes and hair bulbs. Acute and chronic pneumonitis is also a manifestation of the damage that methotrexate

can cause. Liver damage is another side effect of the use of the drug as chemotherapy. All in all, with high doses, most organ systems are not spared. This is because the drug inhibits DNA synthesis (Heyn, Krivit, Nesbit, & Sharp, 1976).

**Radiation.** Radiotherapy involves both external beam radiotherapy and brachytherapy. The type of radiation used depends on the type of tumor and the location within the body. The dose depends on whether the therapy is curative or palliative, the size of the tumor, the radiosensitivity of the tumor, and the amount of damage that can be delivered to the surrounding normal tissue. The effects that happen to the body after radiation happen to the organs that are in the irradiated path. The effects increase with the volume of the tissue treated with the radiation beams (Bristow & Hill, 2005).

Conformal radiotherapy uses three dimensional treatment planning using a series of radiation beams streaming from different angles to maximize the effect of the treatment. The tumor is localized by MRI imaging or a CT scan before the radiotherapy is performed. The energy and radiation beams are then chosen (Bristow & Hill, 2005).

**Tumor markers.** If metastases has not occurred, the patient's chance for survival and for a cure increases greatly. Most of the time, metastases has already occurred before the tumor is detected, so the patient's chance for a cure greatly decreases. Preventive efforts that people use include: mammography for breast cancer, pap smears for cervical cancer, blood detection in the stool for bowel cancer, and physical examination for prostate cancer (Nakamura & Wu, 1997).

Tumor markers were at one time thought to be useful for detection of disease and for early prognostic information. This was because they were shown to be increased in the blood stream several months before symptoms showed up. The tumor marker CEA

was used to look for a colorectal carcinoma in men. It was discovered that the tumor markers did not have enough of a degree of specificity for screening. For example, CEA is also found in the serum of patients with other malignant and nonmalignant illnesses (Nakamura & Wu, 1997).

A very important way tumor markers can be used is to monitor the course of a disease. Tumor marker levels will show whether the disease is in remission or relapsing. Other procedures lack the sensitivity and convenience that the tumor markers have. Tumor markers work better for monitoring the disease because one does not require the same level of specificity as is needed in diagnosis. Tumor markers also provide the information for choosing the best drug for treatment of the cancer (Nakamura & Wu, 1997).

**Major classifications of tumor markers.** A molecule that can be identified with malignant transformation, proliferation, dedifferentiation, and metastases can be used as a tumor marker. The value of a tumor marker is based on its specificity and sensitivity. It has become more popular in recent years to use tumor markers to select for appropriate treatments of tumors (Nakamura & Wu, 1997).

Some major classes of tumor markers are enzymes, isoenzymes, and proteins. They are used today in combination with other tumor markers in multiple marker format. Some enzymes that are used as tumor markers are ribonucleases, prostatic acid phosphatases, and lysozymes. Ribonucleases are used to find the cancer of the pancreas, breast, colon, liver, lung, and stomach. Phostatic acid phosphatases are used to find prostate carcinomas at a late stage. Lysozymes are used to find colon cancer, monocytic and myelomonocytic leukemias (Nakamura & Wu, 1997).

Isoenzymes are another class of tumor markers. Aldolase B, type IV collagen-degrading enzyme, and CK-BB are all types of isoenzyme tumor markers. Aldolase B will show an increase when there is metastatic liver cancer. Type IV collagen-degrading enzyme will increase when there is a detection of metastases of hepatocellular carcinoma, and CK-BB will increase when there is an adenocarcinoma of the prostate, lung, and stomach. CK-BB is not a very specific isoenzyme (Nakamura & Wu, 1997).

Carcinoembryonic proteins are another class of tumor markers. CEA, AFP, and SP<sub>1</sub> carcinoma proteins are examples of this class of tumor marker. CEA is a tumor marker that shows up when there is cancer of the gastrointestinal tract. AFP is a carcinoembryonic protein that increases when there are hepatoma and yolk sac tumors. And lastly, SP<sub>1</sub> increases when there is a trophoblastic tumor (Nakamura & Wu, 1997).

## Materials and Methods

Two of the kits used in this project for the ELISA assays carcinoembryonic antigen (CEA) and neuron specific enolase (NSE) were acquired from Diagnostic Automation, Inc (Calabasas, CA). The third kit for the ELISA assay CYFRA 21-1 was acquired from Fujirebio Diagnostic, Inc (Immuno-Biological Laboratories, Inc). All the solutions that were used were prepared from using diluents present in the kits. Tests were performed using ELISA assays. Statistical analyses were performed using SPSS version 22 statistical software. The samples were tested for the specific antigen at the hospitals of their origin. Permission for this study was granted by the University of Southern Mississippi Institutional Review Board to ensure adherence to stipulated criteria (Appendix A).

Patient sera samples were obtained from area hospitals with only a sample code number and the cancer diagnosis provided. Normal samples from healthy subjects were also obtained from area hospitals. All procedures protecting the confidentiality of the patient were followed. No information regarding the identification of the patient was released by the hospitals involved. Aseptic techniques were used at all times with the samples. Samples were collected by hospital personnel at the respective hospitals, allowed to clot, and were separated before being frozen and packaged in plastic tubes for transport. Before testing, all of the samples were sorted into test tube racks and allowed to reach room temperature by soaking in a water bath at approximately 25°C.

Patient samples were classified by the hospital pathologists as either cancerous or cancer free. This diagnosis was provided for comparison only (see table 1). There were 264 healthy control subjects tested for CEA. There were 200 healthy control subjects tested for CYFRA 21-1, and there were 80 healthy control subjects tested for NSE.

There were testing procedures followed for each assay (CEA, CYFRA 21-1, and NSE). The results of the assays performed were read with a Beckman Coulter AD 340 (Beckman-Coulter, Brea, CA, USA) microplate reader.

Table 1

Patient Sample Classification

Number of Samples	Cancer Diagnosis
76	Cancerous
562	Cancer Free
Total number of Patients: 638	

**CEA ELISA Assay Kit**

The kit's reference number was 5201-16, and the lot# was DA314050802. The kits came from Diagnostic Automation/Cortez Diagnostics, Inc. (Calabasas, CA, USA). Materials also required that did not come in the kits were disposable tips, pipettors of 25 uL and 100 uL, a microwell reader, and deionized water for blanks.

The CEA quantitative test kit is based on a solid phase enzyme-linked immunosorbent assay with a detection range of 0-120 ng/mL. The test requires 50 uL of serum, and it performs with a specificity of 95% and sensitivity of 1.0 ng/mL. The assay system utilizes one monoclonal anti-CEA antibody for solid phase immobilization and another mouse monoclonal anti-CEA antibody in the antibody-enzyme conjugate solution. The standards and the testing specimens were added to the CEA antibody coated microtiter wells. The CEA antibody labeled with horseradish peroxidase (conjugate) was added. If human CEA was present in the specimen, it would combine

with the antibody on the well and the antibody conjugate. The solution was then washed with the wash buffer, which removed any unbound conjugate. The TMB solution was then added. A colorimetric reaction occurs whose final intensity reveals the concentration of CEA present.

When preparing the assay, all the reagents and samples were brought to room temperature (~25°C) and gently mixed. The wash buffer was prepared by adding 15 mL of the washing buffer into 735 mL of distilled water in a large flask. The mixture was capped and inverted several times to mix. The wash buffer was then poured into the solution bottle. Blanks (deionized water), calibration solutions, and controls were run in duplicate in the first 14 wells of each plate. The remaining wells contained serum samples and extra controls. A data sheet was kept to identify samples, calibrators, and controls with their locations. The procedure is outlined in figure 1 as follows.

Figure 1

#### CEA ELISA Assay Kit Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 50 uL of standard, specimens, and controls into appropriate wells.
3. Dispense 100 uL of enzyme conjugate reagent to each well.
4. Thoroughly mix for 10 seconds. It is very important to have complete mixing in this step.
5. Incubate at room temperature (18-22°C) for 60 minutes.
6. Remove the incubation mixture by emptying plate content into a waste container.
7. Rinse and empty the microtiter wells 5 times with washing buffer (1X).
8. Strike the wells sharply onto absorbent paper or paper towels to remove all



residual water droplets.

9. Dispense 100 uL of TMB substrate into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop the reaction by adding 100 uL of stop solution to each well.
12. Gently fix for 30 seconds to ensure that the blue color completely changes to yellow.
13. Read optical density at 450 nm with a microtiter plate reader within 15 minutes

### **NSE ELISA Assay Kit**

The kit's reference number was 6334-16, and the lot# was DA314050901. The kit came from Diagnostic Automation/Cortez Diagnostics, Inc. Materials also required that did not come in the kit were disposable tips, pipettors of 25 uL and 100 uL, a microwell reader, and deionized water for blanks.

The NSE quantitative test kit is based on a solid phase enzyme-linked immunosorbent assay with a detection range of 0-120 ng/mL. The test requires 15 uL of serum, and it performs with a specificity of 98.7% and sensitivity of 1.5 ng/mL (as recorded by Diagnostic Automation/Cortez Diagnostics, Inc.). The assay system utilizes one monoclonal anti-NSE antibody for solid phase immobilization and another monoclonal anti-NSE antibody in the antibody-enzyme conjugate solution. The standards and the testing specimens were added to the antibody coated microtiter wells. If human NSE was present in the specimen, then it would combine with the antibody on the well and the antibody conjugate. The solution was then washed with the wash buffer, which removed any unbound conjugate. The amount of bound peroxidase is proportional to the concentration of the NSE present in each sample. After addition of the substrate

and chromogen, the intensity of blue color developed in proportion to the concentration of NSE antigen in the samples.

When preparing the assay, all the reagents and samples were brought to room temperature (~25°C) and gently mixed. The wash buffer was prepared by adding 15 mL of the washing buffer into 735 mL of distilled water in a large flask. The mixture was capped and inverted several times to mix. The wash buffer was then poured into the solution bottle. Blanks (deionized water), calibration solutions, and controls were run in duplicate in the first 14 wells of each kit. The remaining wells contained serum samples and extra controls. A data sheet was kept to identify samples, calibrators, and controls with their locations. The procedure is outlined in figure 2 as follows.

Figure 2

#### NSE ELIA Assay Kit Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 25 uL of standard, specimens, and controls into appropriate wells.
3. Dispense 100 uL of sample diluent into each well.
4. Thoroughly mix for 10 seconds. It is very important to have complete mixing in this step.
5. Incubate at room temperature (18-22°C) for 30 minutes.
6. Remove the incubation mixture by emptying plate content into a waste container.
7. Rinse and empty the microtiter wells 5 times with washing buffer (1X).
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 uL of Enzyme Conjugate Reagent into each well. Gently mix for 5

seconds.

10. Incubate at room temperature for 30 minutes.
11. Remove the incubation mixture by flicking plate contents into a waste container.
12. Rinse and flick the microtiter wells 4 times with washing buffer (1X).
13. Strike the wells sharply onto absorbent paper to remove residual water droplets.
14. Dispense 100 uL of TMB substrate into each well. Gentle mix for 5 seconds.
15. Incubate at room temperature for 20 minutes.
16. Stop the reaction by adding 100 uL of stop solution to each well.
17. Gently fix for 30 seconds to ensure that the blue color completely changes to yellow.
18. Read optical density at 450 nm with a microtiter plate reader within 30 minutes.

#### **CYFRA 21-1 ELISA Assay Kit**

The kit's number was 211-10, and the lot# was 34112:1. The kits came from Fujirebio Diagnostic, Inc (Seguin, TX, USA). Materials also required that did not come in the kits were disposable tips, pipettors of 25 uL and 100 uL, a microwell reader, and deionized water for blanks.

The CYFRA 21-1 quantitative test kit is based on a solid phase enzyme-linked immunosorbent assay with a detection range of 0.5-50 ng/mL. The test sensitivity and specificity can be seen below in table 2. The assay system utilizes one monoclonal anti-CYFRA 21-1 antibody for solid phase immobilization and another mouse monoclonal anti-CYFRA 21-1 antibody in the antibody-enzyme conjugate solution. The standards and the testing specimens were added to the CYFRA 21-1 antibody coated microtiter wells. The CYFRA 21-1 antibody labeled with horseradish peroxidase (conjugate) was

added. If human CYFRA 21-1 was present in the specimen, then it would combine with the antibody on the well and the antibody conjugate. The solution was then washed with the wash buffer, which removed any unbound conjugate. The TMB solution was then added. A colorimetric reaction occurs whose final intensity reveals the concentration of CYFRA 21-1 present.

When preparing the assay, all the reagents and samples were brought to room temperature (~25°C) and gently mixed. The wash buffer was prepared by adding 50 mL of the washing buffer into 1200 mL of distilled water in a large flask. The mixture was capped and inverted several times to mix. The wash buffer was then poured into the solution bottle. Blanks (deionized water), calibration solutions, and controls were run in duplicate in the first 14 wells of each kit. The remaining wells contained serum samples and extra controls. A data sheet was kept to identify samples, calibrators, and controls with their locations. The procedure is outlined in figure 3 as follows.

Figure 3

#### CYFRA 21-1 ELISA Assay Kit Procedure

1. Prepare CYFRA 21-1 calibrators and controls by adding 1 mL of distilled water to each vial. Allow to stand for at least 15 minutes.
2. Prepare wash solution by adding 50 mL of wash concentration to 1200 mL of distilled water.
3. Prepare antibody solution by mixing 50 uL of tracer, HRP anti-CYFRA 21-1 with 1 mL of Biotin Anti-CYFRA 21-1 per strip.
4. Wash each well once with the wash solution.
5. Add calibrators' controls and samples by putting 50 uL in each well. The mixing

of samples using electric vibration mixers must be limited to a maximum of 1 second.

6. Add antibody solution- 100 uL in each well.
7. Incubate for 1 hour.
8. Wash each well 6 times with wash solution.
9. Add TMB HRP- substrate- 100 uL in each well.
10. Incubate for 30 minutes.
11. Read absorbance at 620 nm.

Table 2

Specificity and Sensitivity per % concentration

% increase in CYFRA 21-1 Concentration	% Sensitivity	% Specificity
30	52.9	84.3
40	48.2	85.6
50	45.9	87.3
60	44.7	88.2
70	43.5	89.5

## Results

Over the course of the project, there were quality control samples incorporated into the assays to determine within-run and between-run precision (Table 3-4). NSE, CEA, and CYFRA 21-1 all had controls run to determine the precision. These controls had a known amount of antigen incorporated into the sample. This determined if the assay was running correctly. The within-run precision was excellent, being under 10% for the percent coefficient of variation for NSE at 2.78%, CYFRA 21-1 at 6.27%, and CEA at 6.78%. The between run precision was also excellent for the percent coefficient of variation of CYFRA 21-1 at 6.16% and CEA at 8.33%. The between run precision was good for NSE with a percent coefficient of variation of 15.37%, being that it was still under 20%.

Serial dilutions of patient samples were used to determine the linearity of the assays (Table 5, Graph 1-3). These results indicate excellent linearity with  $R^2$  values between 0.94 and 0.99.

The minimum concentration each assay was able to detect was determined by calculating the analytical sensitivity (Table 6). The analytical sensitivity of NSE was determined by analyzing 10 replicates and calculating the mean (+/-) two standard deviations ( $\bar{X} \pm 2SD$ ). This revealed a range of 6.10-7.02. The ranges for CYFRA 21-1 and CEA were determined the same way. CYFRA 21-1 had a cut off value of 0.07, determined by analyzing 20 replicates, and CEA had a cut off value of 0.70, also determined by analyzing 20 replicates.

The normal reference intervals ( $\bar{X} \pm 2SD$ ) are the reference intervals that were developed from healthy control subjects. The healthy control subjects were known to have no disease. The normal reference intervals for each of the antigens studied can be

seen in Table 7.

In determining the negative and positive patient results, the manufacturers' cut off values were used. Diagnostic sensitivities of 0.00% (NSE), 18.9% (CYFRA 21-1), and 22.37% (CEA) were obtained (Table 8). Diagnostic sensitivity is the proportion of individuals with a disease who test positive for the disease. The higher the sensitivity the better the test is. Diagnostic specificity is the proportion of individuals without the disease who test negatively for the disease. Diagnostic specificities of 99.39% (NSE), 93.2% (CYFRA 21-1), and 80.43% (CEA) were obtained. Some other parameters that can be evaluated are predictive value (+), predictive value (-), and percent efficiency. Predictive value plus is the fraction of positive tests that are the true positives. Predictive value minus is the fraction of negative tests that are true negatives. The percent efficiency is the fraction of all test results that are either true positives or true negatives.

Table 3

Within Run Assay Precision for NSE, CYFRA 21-1, and CEA

	<b>n</b>	<b>Mean</b>	<b>SD</b>	<b>%CV</b>
NSE control	10	7.55 ng/mL	0.21	2.78
CYFRA 21-1 High Control	20	14.17 ng/mL	0.77	5.41
CYFRA 21-1 Low Control	20	4.41 ng/mL	0.28	6.27
CEA High Control	72	62.64 ng/mL	3.40	5.43
CEA Low control	76	4.44 ng/mL	0.37	8.33

Table 4

Between Run Precision for NSE, CYFRA 21-1, and CEA

	<b>n</b>	<b>Mean</b>	<b>SD</b>	<b>%CV</b>
NSE Control	43	7.87 ng/mL	1.21	15.37
CYFRA 21-1 High Control	76	13.97 ng/mL	0.86	6.16
CYFRA 21-1 Low Control	78	5.45 ng/mL	0.86	6.16
CEA High Control	72	62.64 ng/mL	3.40	5.43
CEA Low Control	76	4.44 ng/mL	0.37	8.33



Table 5

Assay Linearity for NSE, CYFRA 21-1, and CEA

<b>Assay</b>	<b>R squared</b>
NSE	0.997
Cyfra 21-1	0.992
CEA	0.939

Table 6

Analytical Sensitivity for NSE, CYFRA 21-1, and CEA

	<b>n</b>	<b>Mean</b>	<b>SD</b>	<b>Range</b>
<b>NSE</b>	<b>10</b>	<b>6.56 ng/mL</b>	<b>0.23</b>	<b>6.10-7.02</b>
<b>CYFRA 21-1</b>	<b>20</b>	<b>0.01 ng/mL</b>	<b>0.03</b>	<b>0.00-0.07</b>
<b>CEA</b>	<b>20</b>	<b>0.00 ng/mL</b>	<b>0.35</b>	<b>0.00-0.70</b>

Table 7

Normal Reference Intervals for NSE, CYFRA 21-1, and CEA

	<b>N</b>	<b>Minimum</b>	<b>Maximum</b>	<b>Mean</b>	<b>SD</b>	<b>Range</b>
<b>NSE</b>	<b>174</b>	<b>3.62</b>	<b>8.45</b>	<b>6.61</b>	<b>1.31</b>	<b>3.99-9.23</b>
<b>CYFRA 21-1</b>	<b>189</b>	<b>0.00</b>	<b>82.9</b>	<b>2.21</b>	<b>9.36</b>	<b>0.00-20.93</b>
<b>CEA</b>	<b>204</b>	<b>0.00</b>	<b>16.10</b>	<b>2.40</b>	<b>2.63</b>	<b>0.00-7.66</b>

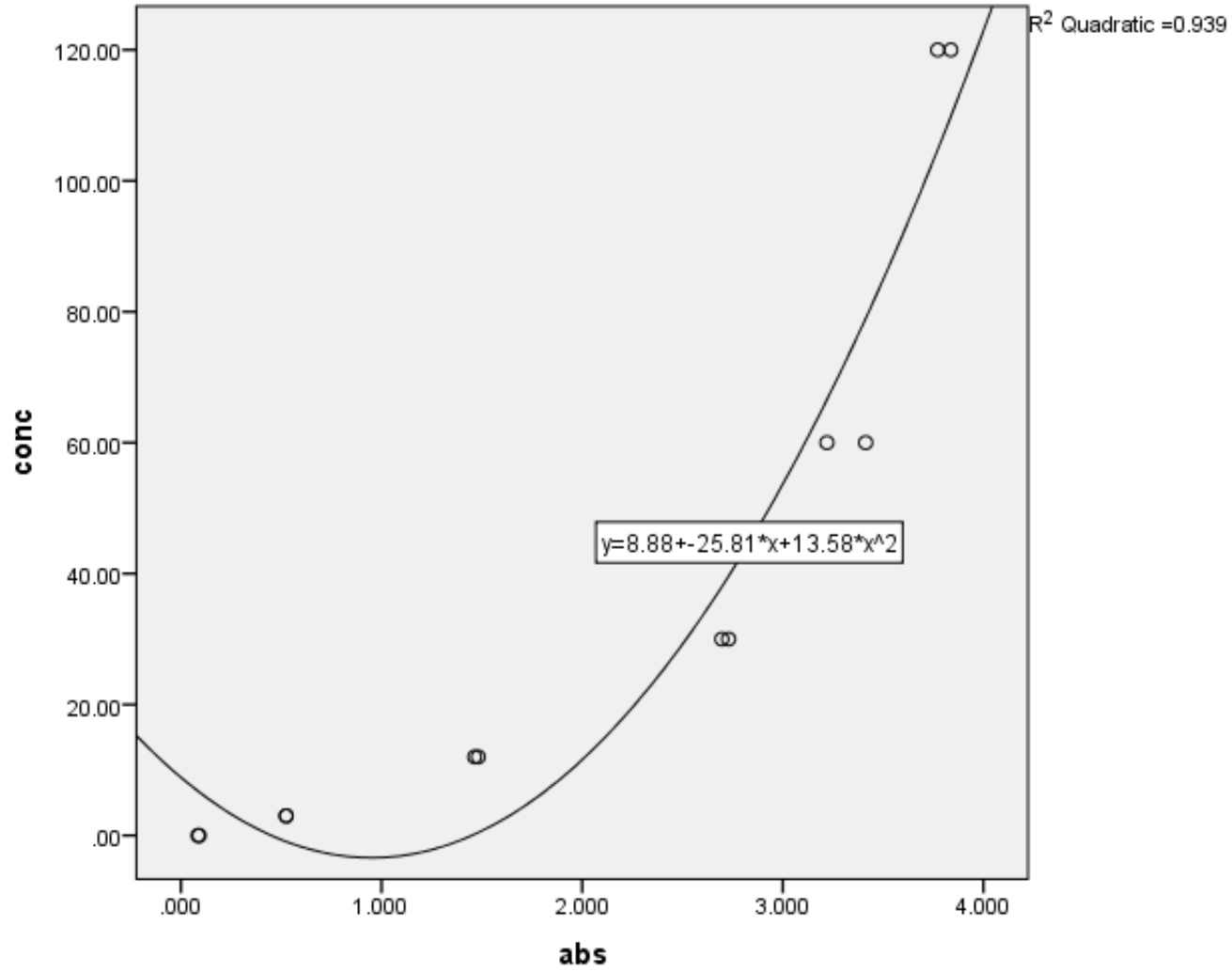
Table 8

Predictive values for NSE, CYFRA 21-1, and CEA in 638 Patients

	<b>Sensitivity (%)</b>	<b>Specificity (%)</b>	<b>PV (+) (%)</b>	<b>PV (-) (%)</b>	<b>Efficiency (%)</b>	<b>Cut-Off (ng/mL)</b>
<b>NSE</b>	0.00	99.39	0.00	87.12	86.65	15.01
<b>CYFRA 21-1</b>	18.9	93.2	28.0	89.1	84.0	1.82
<b>CEA</b>	22.37	80.43	13.39	88.45	73.5	5.01

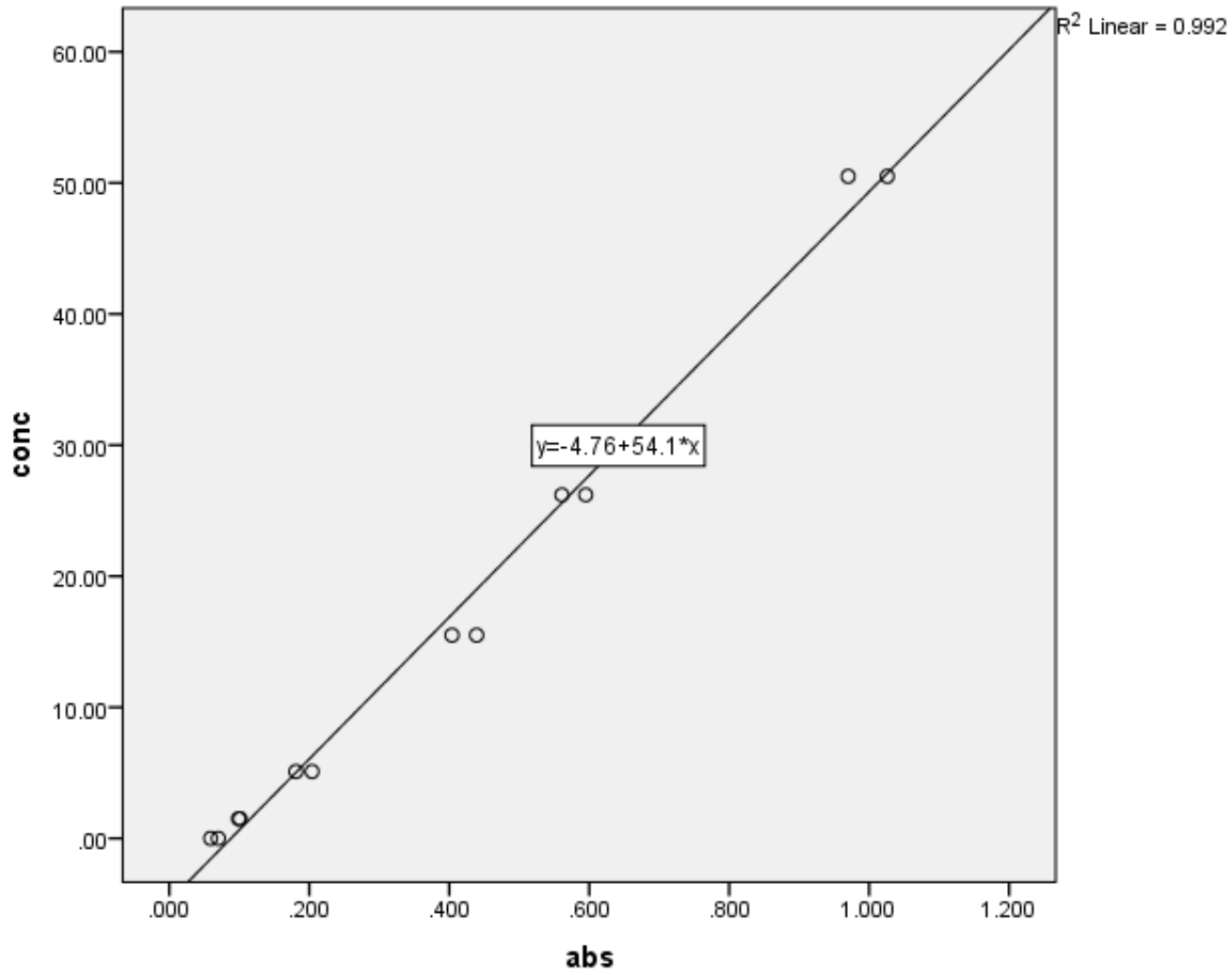
Graph 1

CEA Linearity Plot



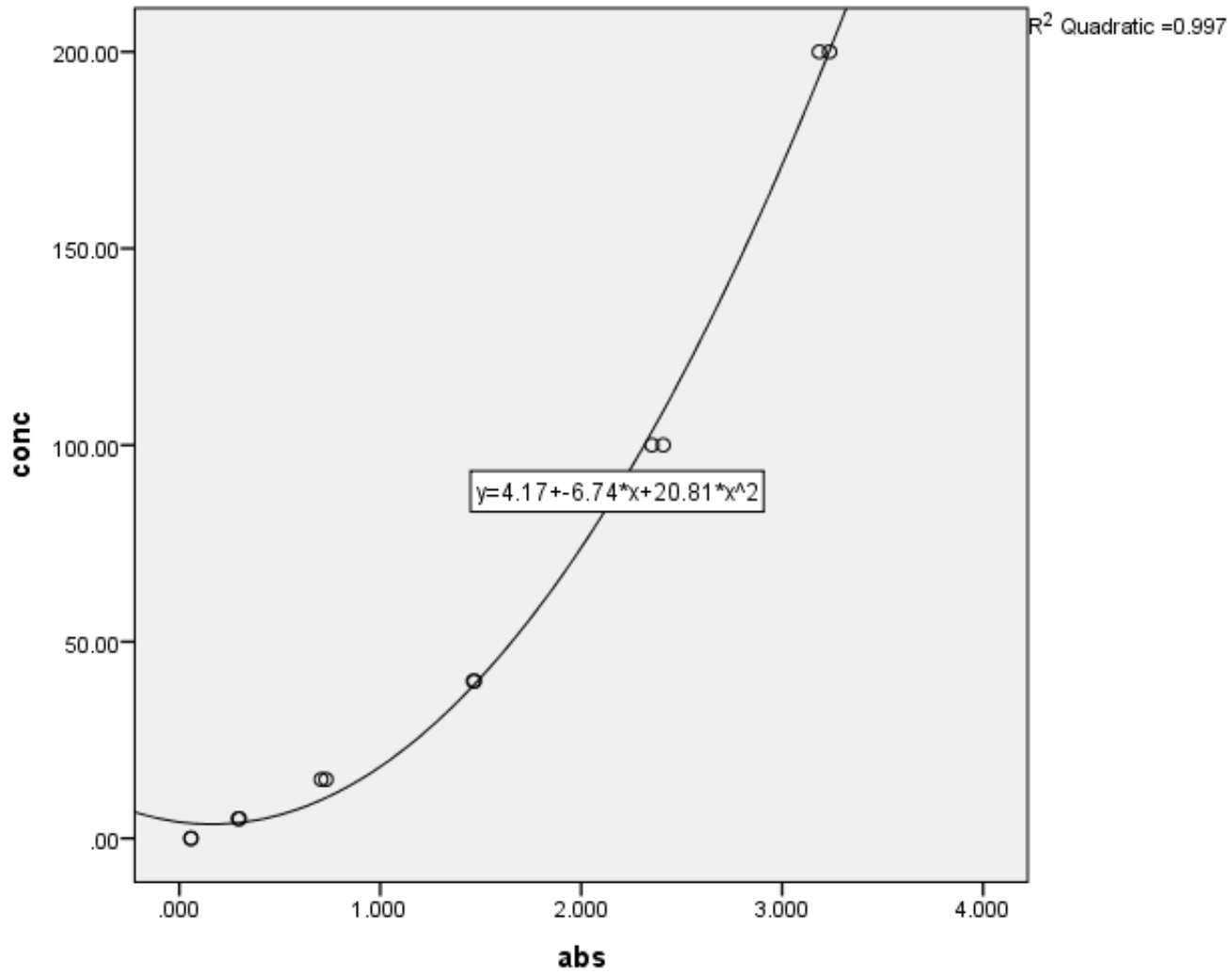
Graph 2

CYFRA 21-1 Linearity Plot



Graph 3

NSE Linearity Plot



## Discussion

The analytical parameters for each of the three testing methods were adequate. The normal reference interval for CYFRA 21-1 was dramatically higher than the reference interval determined by the manufacturer. This is possibly due to geographic location and the mix of healthy adult subjects tested. None of the diagnostic sensitivities were desirable, but of the three examined, CEA was the best predictor of the disease. The sensitivity would be the most important test result because it demonstrates the ability of the assay to diagnose the presence of disease. The diagnostic specificities obtained for the true negatives were excellent, with NSE having the best specificity at 99.39%. This result greatly differed from the 0% found for the sensitivity of NSE. Mathematically, because of the high percent efficiency of NSE at 86.65%, it appeared to be the best predictor of the disease, but a high sensitivity is what is desired. The “cutoff” points used for all three of the markers were those of the manufacturers’. By adjusting the “cutoff” points one could raise the sensitivity, but the specificity would be lowered. For a more accurate study for a given environment and patient mixes, one could use the normal reference intervals as the “cut-off” points.

A strong point of this study is the small number of people that were directly involved in the testing of the samples. This keeps the relative amount of human error minimal. The age of some of the samples is a possible weakness due to potential sample degradation at minus 20°C. To improve the accuracy of the study, a larger number of subjects could be obtained, and the subjects could be acquired from multiple geographic regions. The samples should also be fresh and only thawed once when tested.

## Conclusion

From the data collected, CEA was the most sensitive screen for predicting lung cancer. NSE was the most specific, and CYFRA 21-1 had the next highest sensitivity and specificity. The highest sensitivity is the most important part of a test because it predicts the true positives. CEA, the best predictor of the disease, is one of the oldest tumor markers. It is common in determining other cancers such as colorectal cancer. The CEA subgroup members are cell membrane associated and show a complex expression pattern in normal and cancerous tissues (Hammarstrom, 1999). This is a strong point for the tumor marker because it has the ability to track cancer formation in different areas of the body in different organs. The object of a tumor marker is a non-invasive test to track a patient's health after recovering from cancer. Physicians and researchers are always seeking noninvasive tests like tumor markers to make early diagnosis and track a patient's recovery. The hypothesis that CYFRA 21-1 would be the most accurate and specific predictor of the disease was rejected. CYFRA 21-1 is an independent prognostic factor, though, that is useful in the earlier stages of squamous cell lung cancer (SQC) (Kulpa, 2002). CYFRA 21-1 was not as sensitive to lung cancer for predicting the true positives, and since the object of the game is diagnosing a positive occurrence of the disease, it was not the best predictor.

## References

- Alavanha, M., Catalan, R., Field, W., Klotz, J.B., Krewski, D., Le' Tourneau, E. G., . . . Zielinski, J. M. (2004). Residential Radon and Risk of Lung Cancer A combined Analysis of 7 North American Case-Control Studies. *Epidemiology*, 16(2), 137-145.
- Allison, J. P, Scott, A. M., & Wolchok, J. D. (2012). Monoclonal Antibodies in Cancer Therapy. *Cancer Immunity*, 1-8.
- American Cancer Society. (2014). *Cancer Facts and Figures 2014*, Atlanta: American Cancer Society.
- Bishop, J. (1982). Oncogenes. In *Cancer Biology* (Friedber, E., ed., 1985) 66-76. New York, NY: W.H. Freeman and Company.
- Boyer, M., & Tannock, I. (2005). Cellular and Molecular Basis of Drug treatment for Cancer. In *The Basic Science of Oncology*. 349-375. New York, NY: McGraw-Hill.
- Bristow, G., & Hill, R., (2005). The Scientific Basis of Radiotherapy. In *The Basic Science of Oncology*. 289-321. New York, NY: McGraw-Hill.
- Bristow, G., Harrington, L, Hill, R, & Tannock, I. (2005). *The Basis Science of Oncology*. New York, NY: McGraw-Hill.
- Cairns, J. (1975). The cancer problem. In *Cancer Biology* (Friedber, E., ed., 1985) 4-14. New York, NY: W.H. Freeman and Company.
- Conklin, G. (1949). Cancer and Environment. In *Cancer Biology* (Friedber, E., ed., 1985) 19-23. New York, NY: W.H. Freeman and Company.
- Cooper, G. (1992). Early Detection and Diagnosis. *Elements of Human Cancer*. (Burns,



- J., ed., 1992) 230. Boston, MA: Jones and Bartlett Publishers, Inc.
- Donovan, J., Slingerland, J., & Tannock, I. (2005). Cell Proliferation and Tumor Growth. In *The Basic Science of Oncology*. 167-193. New York, NY: McGraw-Hill.
- Folkman, J. (1976). The Vascularization of Tumors. In *Cancer Biology* (Friedber, E., ed., 1985) 115-124. New York, NY: W.H. Freeman and Company.
- Gerosa, M. Niolato, A., & Foroni, R. (2003). The Role of Gamma Kniffe Radiosurgery in the Treatment of Primary and Metastatic Brain Tumors. *Current Opinion in Oncology: Brain and Nervous System*, 15(3), 188-196.
- Hakem, R., & Harrington, L. (2005). Cell Death. In *The Basic Science of Oncology*. 194-204. New York, NY: McGraw-Hill.
- Hammarstrom, S. (1999). The Carcinoembryonic Antigen (CEA) Family: Structures, Suggested Functions and Expression in Normal and Malignant Tissues. *Seminars in Cancer Biology*, 9(2), 67-81.
- Hellerstedt, B. A., & Pienta, K. J. (2002). The Current State of Hormonal Therapy for Prostate Cancer. *A Cancer journal for Clinicians*, 52, 154-179.
- Heyn, R., Krivit, W., Nesbit, M., & Sharp, H (1976). Acute and Chronic Effects of Methotrexate on Hepatic, Pulmonary, and Skeletal Systems. *Cancer*, 37(2), 1048-1054.
- Hughes, J.M., & Weill, H. (1991). Asbestosis is a Precursor of Asbestos Related Lung Cancer: Results of a Prospective Mortality Study. *British Journal of Industrial Medicine*, 48(4), 229-233.

- Hunter, T. (1984). The Proteins of Oncogenes. In *Cancer Biology* (Friedber, E., ed., 1985) 88-97. New York, NY: W.H. Freeman and Company.
- Jansson, M.D., & Lund, A.H. (2012) MicroRNA and Cancer. *Molecular Oncology*, 6(6), 590-610.
- Kulpa, J., Wojcik, E., Reinfuss, M., & Kolodziejcki, L. (2002). Carcinembryonic Antigen, Squamous Cell Carcinoma Antigen, Cyfra 21-1, and Neuron- Specific Enolase in Squamous Cell Lung Cancer Patients. *Clinical Chemistry*. 1931-1937.
- Boulter, P.S. (1967). Methotrexate in Cancer. *British Medical Journal*, 1(5539), 553.
- McGill, M., & McGlade, C. (2005). Cellular Signaling. In *The Basic Science of Oncology*. 142-166. New York, NY: McGraw-Hill.
- Nicolson, G. (1979). Cancer Metastasis. In *Cancer Biology* (Friedber, E., ed., 1985) 138-148. New York, NY: W.H. Freeman and Company.
- Oster, S., Penn, L., & Stambolic, V. (2005). Oncogenes and Tumor Suppressor Genes. In *The Basic Science of Oncology*. 123-141. New York, NY: McGraw-Hill.
- Przybytkowski, E., Lenkiewicz, E., Barrett, M. T., Klein, K., Nabavi, S., Greenwoodand, . . . Basik, M. (2014). Chromosome- Breakage Genomic Instability and Chromothripsis in Breast Cancer. *BMC Genomics*, 15(579), 1-15.
- Richarson, C. (2005). Viruses and Cancer. In *The Basic Science of Oncology*. 114. New York, NY: McGraw-Hill.
- Sherar, M., (2005). Imaging in Oncology. In *The Basic Science of Oncology*. 249-260. New York, NY: McGraw-Hill.

- Schneider, P. M., Metzger, R., Brabender, J., Boehm, S., Luebke, T., Zarghooni, K., ...  
Hoelscher, A. H. (2002). Lung Cancer. *Tumor Markers Physiology, Pathobiology, Technology, and Clinical Applications* (Diamandis, E. P., Et al. ed.) 290-292, American Association for Clinical Chemistry.
- Temin, H. (1972). RNA- Directed DNA Synthesis. In *Cancer Biology* (Friedber, E., ed., 1985) 56-65. New York, NY: W.H. Freeman and Company.
- Tufts University. (2007). One Reason Why Chromosomes Break, Often Leading To Cancer. *Science Daily*. Retrieved from [www.sciencedaily.com/releases/2007/08/070803091034.htm](http://www.sciencedaily.com/releases/2007/08/070803091034.htm)
- Upton, A. (1982). The Biological Effect of Low-Level Ionizing Radiation. In *Cancer Biology* (Friedber, E., ed., 1985) 24-32. New York, NY: W.H. Freeman and Company.
- Wu, J. (1997). Clinical Applications. In *Human Circulating Tumor Markers*. (Wu, J., Nakamura, R., ed.) 21-35. Chicago, IL: The American Society of Clinical Pathologists.
- Wu, J. (1997). Types of Tumor Markers. In *Human Circulating Tumor Markers*. (Wu, J., Nakamura, R., ed.) 37-71. Chicago, IL: The American Society of Clinical Pathologists.

## Appendices

### Appendix A



**INSTITUTIONAL REVIEW BOARD**  
118 College Drive #5147 | Hattiesburg, MS 39406-0001  
Phone: 601.266.6820 | Fax: 601.266.4377 | www.usm.edu/irb

#### NOTICE OF COMMITTEE ACTION

The project has been reviewed by The University of Southern Mississippi Institutional Review Board in accordance with Federal Drug Administration regulations (21 CFR 26, 111), Department of Health and Human Services (45 CFR Part 46), and university guidelines to ensure adherence to the following criteria:

- The risks to subjects are minimized.
- The risks to subjects are reasonable in relation to the anticipated benefits.
- The selection of subjects is equitable.
- Informed consent is adequate and appropriately documented.
- Where appropriate, the research plan makes adequate provisions for monitoring the data collected to ensure the safety of the subjects.
- Where appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of all data.
- Appropriate additional safeguards have been included to protect vulnerable subjects.
- Any unanticipated, serious, or continuing problems encountered regarding risks to subjects must be reported immediately, but not later than 10 days following the event. This should be reported to the IRB Office via the "Adverse Effect Report Form".
- If approved, the maximum period of approval is limited to twelve months.  
Projects that exceed this period must submit an application for renewal or continuation.

PROTOCOL NUMBER: **13042901**  
PROJECT TITLE: **A Comparison of CYFRA 21-1 and NSE with CEA  
for the Serodiagnosis of Lung Cancer**  
PROJECT TYPE: **New Project**  
RESEARCHER(S): **Margot Hall, Ph.D.**  
COLLEGE/DIVISION: **College of Health**  
DEPARTMENT: **Medical Laboratory Science**  
FUNDING AGENCY/SPONSOR: **N/A**  
IRB COMMITTEE ACTION: **Expedited Review Approval**  
PERIOD OF APPROVAL: **04/29/2013 to 04/28/2014**

**Lawrence A. Hosman, Ph.D.**  
**Institutional Review Board**

## Appendix B

**Human Subjects Research Application**  
**The University of Southern Mississippi**  
**Institutional Review Board**  
 irb@usm.edu

Name Margot Hall, Ph.D. Phone 601-266-4912  
 or 266-4908  
 E-Mail Address Margot.Hall@usm.edu Campus ID # W300523

Mailing Address (address to receive information regarding this application):

118 College Drive #5134 Hattiesburg, MS 39406.  
 Street City State Zip Code

College/Division College of Health Dept. Medical Laboratory Science

Department Box # 5134 Phone 601-266-4908

Title A Comparison of Cyfra 21-1 and NSE with CEA  
for the Serodiagnosis of Lung Cancer.

Funding Agencies or Research Sponsors NA

Grant Number (when applicable) \_\_\_\_\_

New Project

Dissertation  Thesis

Renewal or Continuation: Protocol # \_\_\_\_\_

Change in Previously Approved Project: Protocol # \_\_\_\_\_

MARGOT HALL, PH.D. Margot Hall, Ph.D. 4/22/13.  
 Researcher Name (type) DATE

Advisor Name (if applicable) (type)

Mary Lux Mary Lux 4/24/13  
 Department Chair Name (type)