



Abstract

Epilepsy is a chronic neurological disorder affecting over 50 million individuals worldwide, including approximately 1.2% of the U.S. population, with significant representation among women of childbearing age. Managing epilepsy during pregnancy poses significant challenges as physiological and hormonal fluctuations can alter the pharmacokinetics of anti-seizure medications, potentially compromising both maternal and fetal health. During pregnancy, maternal estradiol levels rise significantly and remain elevated, influencing the metabolism of anti-seizure medications due to hormonal changes. This study employs the enzyme-linked immunosorbent assay (ELISA) method to quantify estradiol levels in EDTA plasma samples from pregnant women with epilepsy. Monitoring these levels is integral to building pharmacokinetic models to adjust medication dosages, minimizing seizure risks, and promoting healthy fetal outcomes. The aim of this research is to assess the effectiveness of the ELISA assay for measuring estradiol levels compared to traditional send-out tests, assessing its potential for faster, more cost-effective in-house hormone level monitoring. However, the findings reveal that EDTA-treated samples are incompatible with the ELISA assay for measuring estradiol levels due to the possible interference with antigen-antibody binding, enzymatic activity or assay linearity.

Introduction

Epilepsy is a chronic neurological disorder characterized by recurrent seizures, which involve involuntary body movements and can lead to loss of consciousness and bladder or bowel control. In the United States, about 1.2% of the population has an active diagnosis of epilepsy, including over half a million women of childbearing age. Epilepsy often requires continuous treatment to manage seizure activity and minimize risks to both mother and fetus. This poses challenges for women with epilepsy because the most pressing concerns for them is balancing the risks of seizure recurrence against the potential for anti-seizure medications to cause birth defects.

Pregnancy introduces significant physiological, metabolic, and hormonal changes that can alter the pharmacokinetics of medications. Fluctuations in sex hormones, particularly estradiol, can influence the metabolism of anti-seizure medications, potentially affecting their effectiveness and safety. Understanding this relationship is crucial for adjusting drug dosages and creating personalized treatment plans for women with epilepsy who are trying to conceive or are pregnant. The aim of this research is to measure estradiol levels in women with epilepsy who are trying to conceive using the Estradiol ELISA Kit (Cayman Chemical), which quantifies estradiol in plasma samples. Monitoring these levels is essential for developing pharmacokinetic models that can adjust anti-seizure medications during pregnancy.

Method

In this research, estradiol levels in patient samples are measured using a competitive enzyme-linked immunosorbent assay (ELISA). The process follows these steps:

1. Estradiol in the sample competes with a fixed amount of estradiol acetylcholinesterase (AChE) conjugate (Estradiol AChE Tracer) for binding to estradiol-specific antibodies that are added to each well.
2. Once binding occurs, estradiol-antibody complexes are captured by a secondary antibody (mouse monoclonal anti-rabbit IgG), which has been pre-coated onto the microplate.
3. Unbound substances are removed through a washing step, leaving only the antibody-bound complexes.
4. Ellman's Reagent that contains a substrate for AChE is added to each well to trigger a color-producing enzymatic reaction.

The intensity of the yellow color produced is measured spectrophotometrically at 414 nm using a microplate reader. The color intensity is proportional to the amount of Estradiol AChE Tracer bound to the well and inversely proportional to the amount of native estradiol in the sample. This inverse relationship allows estradiol concentration to be quantified by comparing the color intensity to a standard curve.

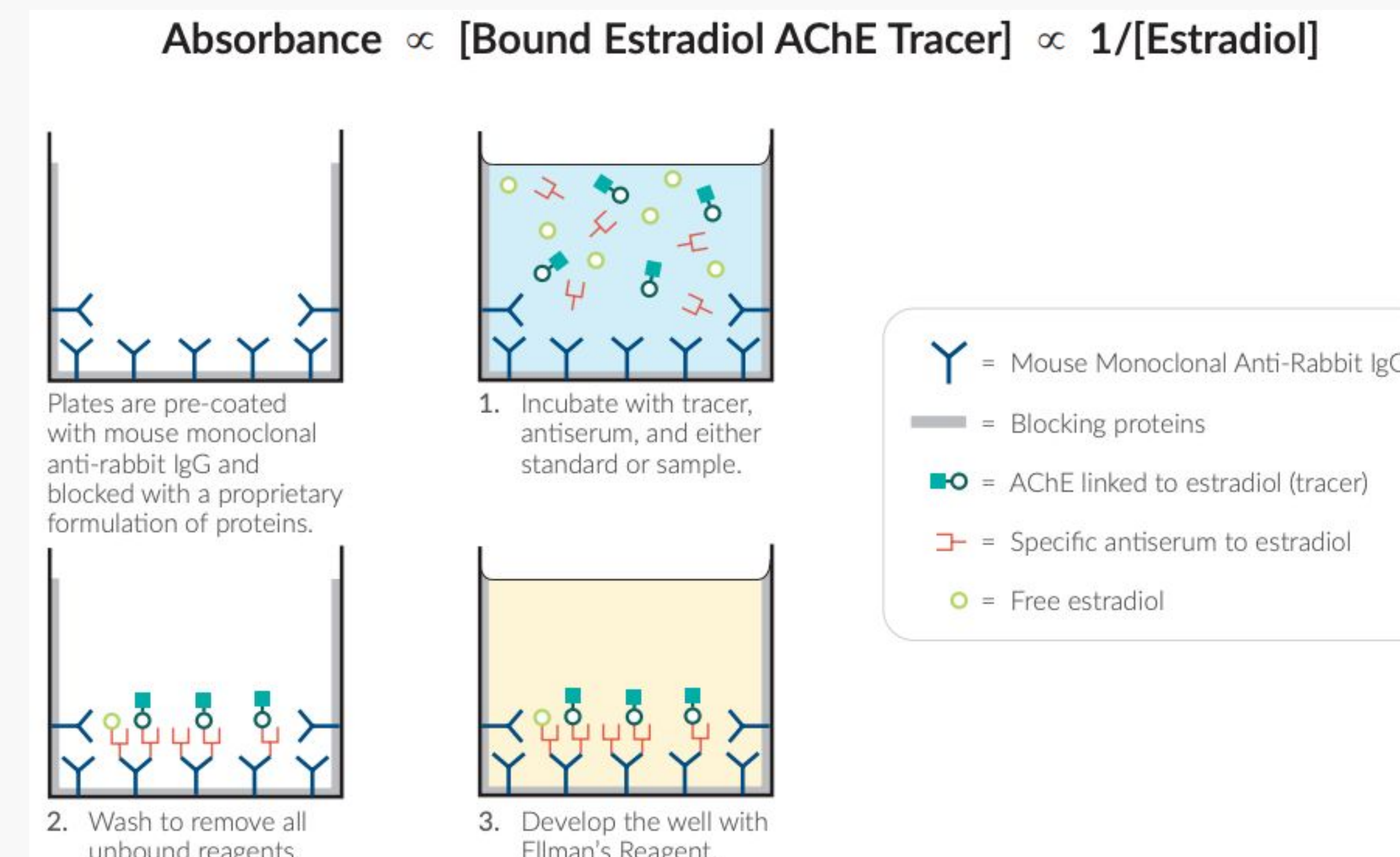


Figure 1. Schematic of the ELISA (from Cayman Chemical package insert)

Conclusion

Choosing the correct specimen type is crucial for obtaining accurate and reproducible results. Careful planning in study design, along with a thorough understanding of specimen preparation and handling, is essential for successful testing. Future research can be done to compare hormone analysis results between EDTA-treated plasma and serum samples to determine whether the issue lies with the ELISA Cayman Chemical kit method or the specimen type.

Results

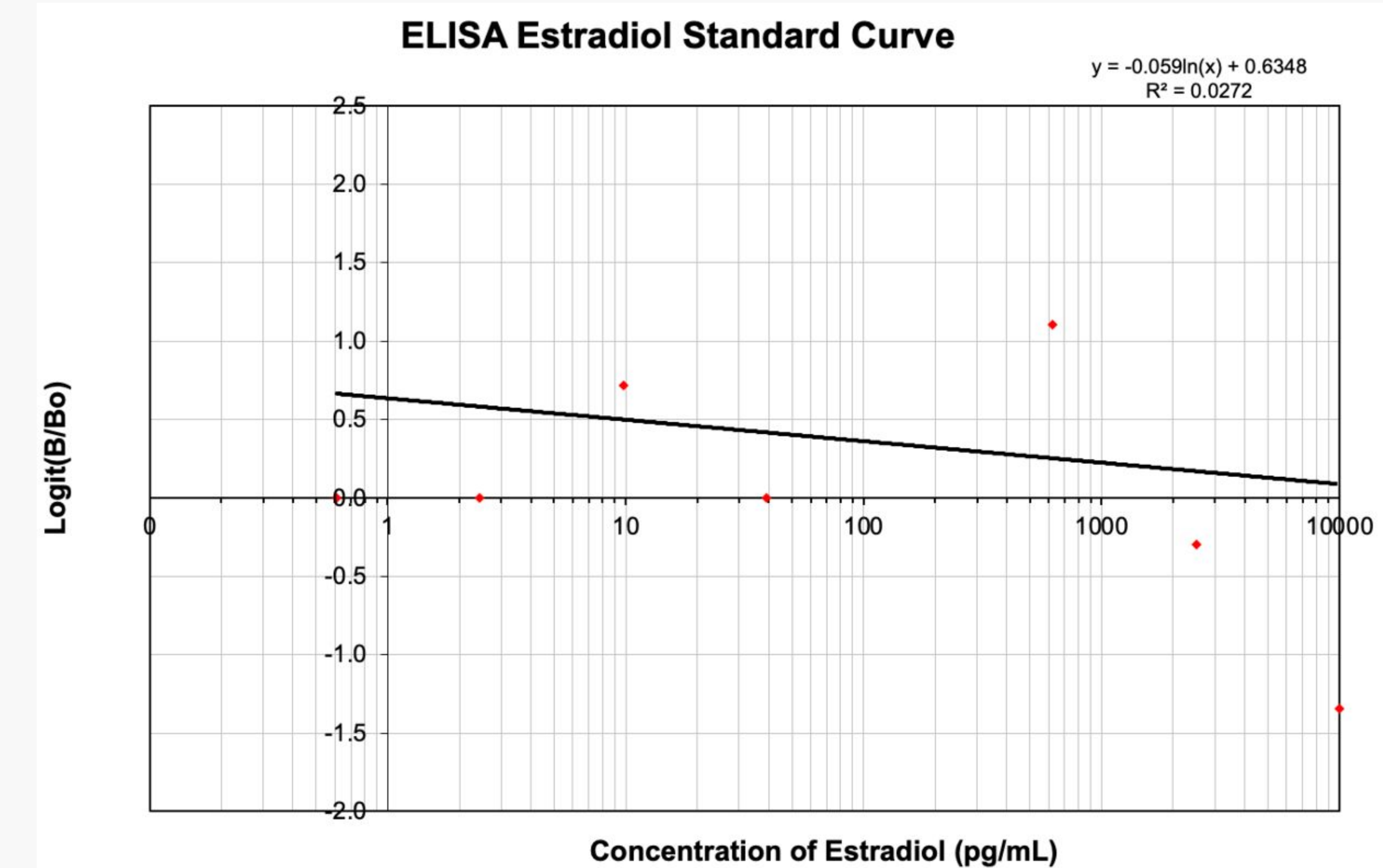


Figure 2. ELISA Estradiol Standard Curve

Discussion

The assay to measure estradiol in human EDTA blank plasma and quality controls of varying estradiol concentrations was unsuccessful likely due to interference from EDTA that disrupted the enzyme-substrate reaction or antibody binding, causing high absorbance values across samples with different estradiol concentrations. Additionally, the age of the kits and potential procedural errors may have contributed to the lack of a valid calibration curve and poor data linearity. In research and clinical testing, selecting the appropriate specimen type is important for obtaining reliable and accurate results. Study design must carefully consider the sample type for each assay to ensure compatibility and avoid interference. While plasma is often preferred for its convenience (e.g., no gel separation), researchers may overlook how certain additives, like EDTA, can affect downstream testing. EDTA-treated plasma is commonly used for DNA analysis, but as seen in this study, it could interfere with hormone measurements, making it ineffective for ELISA-based assays.

Acknowledgement

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References

