Fluorescent Probe Tagging of Androstenedione to Effectively Diagnose Endometriosis

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Problem

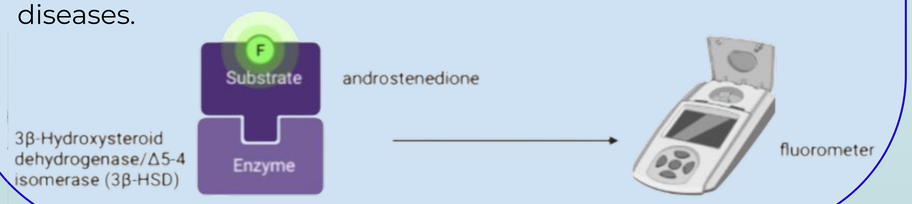
Ten percent of the worlds' women have endometriosis, a gynecological disorder where the lining of the tissue is outside the uterus instead of inside. This leads to **inflammation** and **scarring** around the area. Women with endometriosis are more likely to have **infertility** and trouble getting pregnant. While endometriosis is not a fatal illness, it can cause significant **pain** and **discomfort** to those who are affected. It can take **4-11 years** for endometriosis to be diagnosed after the onset of a patient's symptoms. Additionally, **60%** of endometriosis cases remain **undiagnosed**. Several current techniques for diagnosis are **invasive** and tend to curate **false positives** and negatives.

History and Comparison to Alternatives

Type of Treatment	Laparoscopy	Pelvic Exam	MRI/ CT Scan	Our Solution
Invasiveness	surgical incision	hand insertion into vagina	non-invasive, but utilizes electromagnetic waves	non-invasive
Costliness	$1,700-5,000	$100-250 with no health insurance	$1,800	$20-30
Convenience	has to be done by a professional	done by doctors at clinics	high-tech hospitals, takes 30 min - 1 hr	performed at home
Accuracy	97.68% sensitivity, 79.23% specificity	50% for vaginal endometriosis, misdiagnosis is common	90% accuracy	90-95% accuracy
Restrictions	fast before, no physical activity for 24 hrs after	recommended age over 21	not able for patients with implants	women need to be on their period & have substantial blood flow
Side Effects	swelling, 2 week recovery time, bleeding	discomfort, bleeding	nausea, headache, pain	no side effects
Turnaround Time		up to 3 weeks	1-2 days	2-3 weeks

Detailed Solution

1. **At-Home testing kit:** During their menstrual cycle, patients will place a used tampon into a sterilized **vial** that contains the **anticoagulant** (saline-adenine-glucose -mannitol or SAG-M). The **thermally regulated** kit will then be packaged by the patient and shipped to labs for testing.
2. **Laboratory Testing:** The hormone **DHEAS i**s known to be linked to endometriosis. Basal plasma concentrations of DHEAS are **significantly higher** in endometriosis patients versus controls in the **follicular stage** (the longest stage in the menstrual cycle). Women with endometriosis have a DHEAS level of **4.33 +/- 0.73 µg/ml**, whereas women without the disorder have a DHEAS level of **2.22 +/- 0.55 µg/ml.** DHEAS is formed as the product of the **3B-hSD enzyme** and its substrate (**androstenedione**). By analyzing the amount of substrate, we can figure out the concentration of DHEAS. After tagging androstenedione using **fluorescent** probes, a **fluorometer** will be used to **quantify** the light emitted. We quantify the fluorescence from the light emitted from one DHEA molecule and multiply by the amount of DHEA molecules present in the blood sample. Then, we compare this with the known DHEAS thresholds. To do so, we will utilize **DHEA-Bodipy**, a functional fluorescent DHEAS analog suitable for fluorescence microscopy of living cells in the blood sample. After confirmation of accuracy, patients will be sent the results of their test as well as the likelihood of other diseases.

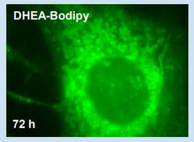


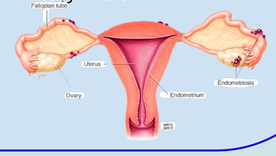
Testing and Benchmarking

Testing: To determine the accuracy of our at-home diagnosis kit, we will test our kit's accuracy in detecting endometriosis by performing **clinical tests** on a large sample group. 50% will have endometriosis, and the other 50% will not. We will evaluate the **specificity** and **sensitivity** of the test and track false positives and negatives by comparing our results to the predetermined groups.
Benchmarking: To diagnose, we are comparing the levels of DHEAS in the patient to known thresholds. The success rate of our kit will be determined by the percent of times it provides an accurate result. The end goal of the testing process is to have a high specificity and high sensitivity.

Ethics

Acquire the facts: The cause of endometriosis has not been determined, but the most likely cause is retrograde menstrual flow.
Alternatives: Current treatments and diagnosis methods to endometriosis include hormone regulation, laparoscopic surgery, or other medical procedures all of which are costly, invasive, or hard to access.
Assessment: A possible solution to the problem of diagnosing endometriosis is to use the enzyme-substrate relationship to tag molecules involved with endometriosis. If the concentration of the product is monitored, then it is possible to determine if the patient has endometriosis or not. Possible risks include the potential for contamination while the patient is retrieving their blood sample.
Action: A hormone that could be used in DHEA-S, which is the product of the 3B-hSD pathway. Research shows that there is an increased concentration of DHEA-S in patients with endometriosis which makes it a good option for tracking. Once the levels of DHEA-S have been determined from analyzing a blood sample, it can be compared to the known thresholds to determine the diagnosis.





Technical Challenges

1. Finding a biomarker that could correlate an explicit, directly causal relationship with endometriosis
2. Finding an effective way to tag androstenedione (the biomarker)
3. Receiving and quantifying the emitted light to compare to threshold DHEAS values
4. Finding the appropriate balance between accessibility and accuracy

References

Almeida Filho, D. P. de, Oliveira, L. J. de, & Amaral, V. F. do. (2008). Accuracy of laparoscopy for assessing patients with endometriosis. *Sao Paulo Medical Journal = Revista Paulista de Medicina*, *126*(6), 305–308. https://doi.org/10.1590/s1516-31802008000600002
Biscione, A., Bernardi, M., Barrasso, G., Verrone, A., Amoruso, N., Starace, A., Bellone, E., Darino, I., Centini, G., & Luisi, S. (n.d.). *Role of DHEA and DHEA-S in the pathogenesis and progression of endometriosis * Introduction * Results.* Retrieved March 26, 2022, from http://seud.org/wp-content/uploads/2018/06/193-Biscione.pdf
Lemcke, S., Hönnscheidt, C., Waschatko, G., Bopp, A., Lütjohann, D., Bertram, N., & Gehrig-Burger, K. (2010). DHEA-Bodipy—A functional fluorescent DHEA analog for live cell imaging. *Molecular and Cellular Endocrinology*, *314*(1), 31–40. https://doi.org/10.1016/j.mce.2009.09.006
Magnetic Resonance Imaging (MRI) | Kaiser Permanente. (n.d.). Healthy.kaiserpermanente.org. Retrieved March 26, 2022, from https://healthy.kaiserpermanente.org/health-wellness/health-encyclopedia/he.magnetic-resonance-imaging-mri.hw214278#:~:text=The%20radiologist%20may%20discuss%20initial
Mayo Clinic. (2019). *Endometriosis - Diagnosis and Treatment - Mayo Clinic.* Mayoclinic.org. https://www.mayoclinic.org/diseases-conditions/endometriosis/diagnosis-treatment/drc-20354661
World Statistics. (2019). Endometriosis World. https://endometriosisworld.weebly.com/world-statistics.html