BME Design-Fall 2021 - Georgia Hancock
Complete Notebook

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Josephine HALL (jrhall3@wisc.edu)

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Team Contact Information

Josephine HALL (jrhall3@wisc.edu) - Oct 19, 2021, 5:43 PM CDT

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Project Description

KARINA BUTTRAM - Dec 14, 2021, 5:38 PM CST

Course Number: BME 200/300

Project Name: Non-Invasive Early Cervical Cancer Screening and Detection

Short Name: Early Detection Cervical Cancer Testing

Project Description/Problem Statement:

Cervical cancer is one of the most common cancers in women and also is one of the most treatable cancers when diagnosed early [1]. Current cervical cancer screenings include Pap smears and HPV (human papillomavirus) tests. Testing methods such as the Pap smear must be collected by a medical professional, as it requires cells to be collected from the surface of the cervix and vagina [3]. While these tests are somewhat successful at detecting cervical cancer [2], they are not easily accessible for people in developing countries and can be an uncomfortable experience. The development of a discrete self-collected urine sample test would increase early cervical cancer detection by providing a cost-effect and culturally sensitive screening option. This device would allow cervical cancer screenings to be easily accessible worldwide, which in turn would prevent many cervical cancer-related deaths.

References

[1] “Cervical cancer,” *World Health Organization*. [Online]. Available: https://www.who.int/health-topics/cervical-cancer#tab=tab_1. [Accessed: 23-Sep-2021].

[2] E. Nkwabong, I. Laure Bessi Badjan, and Z. Sando, “Pap smear accuracy for the diagnosis of cervical precancerous lesions,” *Tropical Doctor*, vol. 49, no. 1, pp. 34–39, 2018.

[3] “HPV and PAP testing,” *National Cancer Institute*, 20-Dec-2019. [Online]. Available: https://www.cancer.gov/types/cervical/pap-hpv-testing-fact-sheet#what-is-cervical-cancer-screening. [Accessed: 23-Sep-2021].

Updated Problem Statement:

Cervical cancer is one of the most common cancers in women and is one of the most treatable cancers when diagnosed early [1]. Current cervical cancer screenings include routine Pap smears and occasional HPV (human papillomavirus) oncoprotein tests using laboratory techniques. A Pap smear must be performed by a medical professional, as it requires cells to be collected from the surface of the cervix and vagina [2]. While these tests are successful at detecting cervical cancer [3], they are uncomfortable and not easily accessible for people in developing countries. The development of a self-collected urine sample test would increase accessibility and allow more cervical cancer screenings to be performed worldwide, which in turn would prevent many cervical cancer-related deaths.

References

[1] “Cervical cancer,” *World Health Organization*. [Online]. Available: https://www.who.int/health-topics/cervical-cancer#tab=tab_1. [Accessed: 23-Sep-2021].

[2] “HPV and PAP testing,” *National Cancer Institute*, 20-Dec-2019. [Online]. Available: https://www.cancer.gov/types/cervical/pap-hpv-testing-fact-sheet#what-is-cervical-cancer-screening. [Accessed: 23-Sep-2021].

[3] E. Nkwabong, I. Laure Bessi Badjan, and Z. Sando, “Pap smear accuracy for the diagnosis of cervical precancerous lesions,” *Tropical Doctor*, vol. 49, no. 1, pp. 34–39, 2018.

About the Client:

Our client, Kebron Zegeye, is a biomedical engineer located in Ethiopia.

9/14/21 - Team Meeting 1

Josephine HALL (jrhall3@wisc.edu) - Sep 27, 2021, 8:57 AM CDT

Title: Team Meeting 1

Date: Sept. 14, 2021

Content by: Cora Williams

Present: Mira Baichoo, Georgia Hancock, Adrienne Simpson, Josephine Hall

Goals:

- Determine client's wants and needs for the project

Content:

- Client did not show
- General team member introductions

Conclusions/action items:

- Schedule another meeting with client
- Continue basic research
- Continue updating lab notebooks

9/19/21 - Team Meeting 2

Josephine HALL (jrhall3@wisc.edu) - Sep 27, 2021, 8:58 AM CDT

Title: Team Meeting 2

Date: Sept. 19, 2021

Content by: Cora Williams

Present: Josephine Hall, Karina Buttram , Cora Williams

Goals:

- Determine client's wants and needs for the project

Content:

- Client did not show
- General team member introductions

Conclusions/action items:

- Schedule another meeting with client
- Continue basic research
- Continue updating lab notebooks

9/22/21 - Team Meeting 3

Cora Williams - Sep 25, 2021, 7:59 PM CDT

Title: Team Meeting 3

Date: Sept. 22, 2021

Content by: Cora Williams

Present: Mira Baichoo, Karina Buttram, Josephine Hall, Georgia Hancock, Adrienne Simpson

Goals:

- Discuss future goals
- Assign group member tasks

Content:

- Question for Advisor
    - Funding?
        - School or us or client?
- Deliverables
    - Client wants us to have a method of testing and have the test work and a physical
    - This won't be able to be done by the end of the semester
        - What deliverables are realistic for the end of the semester to be carried over to the BME 400
- Looked at PDS
    - Separated each section of the PDS for each member of the team

Conclusions/action items:

- Meet with advisor and team
- Complete PDS
- Continue basic research
- Continue updating lab notebooks

9/24/21 - Team Meeting 4

Cora Williams - Sep 25, 2021, 8:04 PM CDT

Title: Team Meeting 4

Date: Sept. 24, 2021

Content by: Cora Williams

Present: Mira Baichoo, Karina Buttram, Josephine Hall, Georgia Hancock, Adrienne Simpson, Cora Williams

Goals:

- Revise PDS as necessary
- Complete progress report
- Determine group member tasks for following week

Content:

- Revised PDS and made lots of revisions
- Completed progress report
- Submitted PDS and progress report to advisor, client, and website
- Determined necessary research and work for next week

Conclusions/action items:

- Continue research
- Work on design matrix
- Determine testing method
- Continue updating lab notebooks

9/29/21 - Team Meeting 5 (Sample Medium Design Matrix)

Cora Williams - Oct 03, 2021, 3:05 PM CDT

Title: Team Meeting 5 (Design Matrix 1 Creation)

Date: Sept. 29, 2021

Content by: Josephine Hall

Present: Josephine Hall, Georgia Hancock, Adrienne Simpson, Cora Williams

Goals:

- Create a design matrix to determine what kind of sample will be collected

Content:

- Team chose the three designs to be blood, saliva, and urine
- Team created a list of requirements and ranked the requirements in order of importance
- Each present member was assigned one to two requirements to define

Conclusions/action items:

- Continue research
- Continue updating lab notebooks
- Meet with advisor on Friday, October 1

10/4/21 - Team Meeting 6 (Collection Device Design Martix)

Cora Williams - Oct 04, 2021, 6:53 PM CDT

Title: Team Meeting 6

Date: Oct. 4, 2021

Content by: Cora Williams

Present: Mira Baichoo, Karina Buttram, Josephine Hall, Georgia Hancock, Adrienne Simpson, Cora Williams

Goals:

- Complete Collection Device Design Matrix
- Determine group member tasks for upcoming week

Content:

- Strip Dip
    - User urinates in a cup and dips the test strip into the collected urine
    - Very little fabrication on our part
- Bean in a Bed
    - User urinates in a cup and draws a portion of the sample into a pipette
    - User deposits a few drops of urine into hole in test packaging
    - Requires more fabrication on our part
- Shewee
    - User urinates into a funnel that is attached to the test compartment
    - Test runs without user interference
    - Biggest fabrication requirements
- Distributed work to all group member
    - Mira, ,Josephine, Georgia, Adrienne, and Cora
        - Design matrix descriptions
    - Karina
        - Funding proposal

Conclusions/action items:

- Continue research
- Work on design matrix descriptions
- Continue updating lab notebooks

10/14/21 - Team Meeting 7

Cora Williams - Oct 18, 2021, 2:10 PM CDT

Title: Team Meeting 7

Date: Oct. 14, 2021

Content by: Cora Williams

Present: Mira Baichoo, Karina Buttram, Josephine Hall, Georgia Hancock, Adrienne Simpson, Cora Williams

Goals:

- Practice preliminary design presentation

Content:

- Distributed work to all group members and ensured that it was an even distribution
- Practiced preliminary design presentation

Conclusions/action items:

- Continue research
- Practice preliminary design presentation independently
- Continue updating lab notebooks

10/19/21 - Team Meeting 8

Cora Williams - Oct 19, 2021, 5:50 PM CDT

Title: Team Meeting 8

Date: Oct. 19, 2021

Content by: Cora Williams

Present: Mira Baichoo, Josephine Hall, Adrienne Simpson, Cora Williams

Goals:

- Finish preliminary deliverables

Content:

- Determined what still needed to be finished before tomorrow
    - Lab Archives
    - Preliminary progress report
- Worked on finishing preliminary deliverables
- Assigned sections of preliminary deliverables to team members to be finished before tomorrow

Conclusions/action items:

- Continue research
- Finish preliminary design deliverables
- Submit preliminary design deliverables
- Continue updating lab notebooks

10/26/21 - Fabrication Team Meeting

Cora Williams - Nov 03, 2021, 10:14 AM CDT

Title: Fabrication Team Meeting

Date: Oct. 26, 2021

Content by: Mira Baichoo

Present: Mira Baichoo, Adrienne Simpson, Georgia Hancock

Goals:

- Make a new SolidWorks design for final design
- Make new SolidWorks file for design 1 (cup)

Content:

- Sat down and made a final design for first 3D print of the final drop test in SolidWorks
- I showed Georgia and Adrienne the cup design I made in SolidWorks and it was approved as a good baseline design since it is not our final design.

Conclusions/action items:

- Set up a meeting with the Makerspace to print our first prototype

10/27/21 - Research Team Meeting

Cora Williams - Nov 03, 2021, 10:22 AM CDT

Title: Research Team Meeting

Date: Oct. 27, 2021

Content by: Cora Williams

Present: Karina Buttram, Josephine Hall, Cora Williams

Goals:

- Discuss recent research findings
- Determine which biomarker we want to test for

Content:

- Discussed recent research findings
    - Karina found information stating how early E6 oncoproteins can be found (20-30 years before cervical cancer)
    - Josie found more information about E5/E6/E7 oncoproteins, how they work, and how they relate to cervical cancer
    - Cora found a competing design that also tests for E6 oncoproteins
- We decided that we are going to test for E5/E6/E7 oncoproteins and potentially an antibody also
    - We need to figure out how to lyse the E5/E6/E7 oncoproteins
    - We need to figure out how to test for E5/E6/E7 oncoproteins
        - We are considering using a peptide sequence
    - We need to determine if there is an antibody that could potentially work
    - We need to determine if the reagents used to test for E5/E6/E7 and the reagents used to test for antibodies will react negatively with each other

Conclusions/action items:

- Continue research
- Continue updating lab notebooks

11/3/21 - Research Team Meeting

Cora Williams - Nov 03, 2021, 9:48 PM CDT

Title: Research Team Meeting

Date: Nov. 3, 2021

Content by: Cora Williams

Present: Karina Buttram, Josephine Hall, Cora Williams

Goals:

- Discuss recent research findings
- Determine what we need to continue researching

Content:

- Discussed recent research findings
    - Karina found the proteins that E6 and E7 oncoproteins bind to
    - Josie found information about antibody concentration levels in vaccinated vs infected women and the peptide sequence that E6 binds to
    - Cora found the proteins that E6 and E7 oncoproteins bind to and the specific functions of E6 and E7
- We decided that we are only going to test for E5/E6/E7 oncoproteins instead of testing for both the oncoproteins and an antibody
    - We need to figure out how to lyse the E5/E6/E7 oncoproteins
    - We need to figure out the peptide sequence that E7 binds to
    - We need to determine if the reagents used to test for E5/E6/E7 and the reagents used to test for antibodies will react negatively with each other
    - We need to determine if there is anything else in urine that will react with the reagents
    - We need to determine what color-changing reactant we are going to use

Conclusions/action items:

- Continue research
- Continue updating lab notebooks

11/11/21 - Research Team Meeting

Cora Williams - Nov 21, 2021, 10:29 PM CST

Title: Research Team Meeting

Date: Nov. 11, 2021

Content by: Cora Williams

Present: Karina Buttram, Josephine Hall, Cora Williams

Goals:

- Discuss recent research findings
- Determine what we need to continue researching

Content:

- Discussed recent research findings
    - Karina found a source that confirms E6 and E7 are found in urine sediment
    - Josie found a peptide sequence that E6 binds to
    - Cora found a peptide sequence that E7 binds to
        - E7 binds to LXCXE motif in pRB, which disrupts pRB-E2F protein complexes that are responsible for tumor suppression
- We discovered that we still have a lot of research to complete before the final presentations
    - We need to figure out how to lyse the E5/E6/E7 oncoproteins
    - We need to determine if the reagents used to test for E5/E6/E7 will react negatively with each other
    - We need to determine what color-changing reactant we are going to use
        - Possibly a bioflourescent?
    - We need to research the false positive rates in the OncoE6 product (competing design)
    - We need to determine if E6/E7 is found in other cancers

Conclusions/action items:

- Continue research
- Continue updating lab notebooks

11/17/21 - Research Team Meeting

Cora Williams - Nov 21, 2021, 10:33 PM CST

Title: Research Team Meeting

Date: Nov. 17, 2021

Content by: Cora Williams

Present: Karina Buttram, Josephine Hall, Cora Williams

Goals:

- Discuss recent research findings
- Determine what we need to continue researching

Content:

- Discussed recent research findings
    - Karina found that bioflourescent dyes will not work for our project because they require lab equipment; however, telerium or selenium might be useful
    - Cora found other cancers caused by HPV also have E6/E7 oncoproteins
- We discovered that we still need to complete a lot of research before the final presentation
    - We need to figure out how to lyse the E5/E6/E7 oncoproteins
    - We need to determine what color-changing reactant we are going to use
        - Possibly telerium or selenium

Conclusions/action items:

- Continue research
- Continue updating lab notebooks

12/1/21 - Team Meeting

Cora Williams - Dec 01, 2021, 8:04 PM CST

Title: Team Meeting

Date: Dec. 1, 2021

Content by: Cora Williams

Present: Karina Buttram, Josephine Hall, Georgia Hancock, Adrienne Simpson, Cora Williams

Goals:

- Finish preliminary deliverables

Content:

- Discussed researched findings
    - We can use the same dye and some of the same antibodies used in a pregnancy test
- Discussed what we still need to research
    - How to coat the antibodies in dye
    - What concentration is needed to test for E6/E7
    - How do we immobilize the antibodies on the test strip

Conclusions/action items:

- Continue research
- Begin final presentation
- Begin final report
- Continue updating lab notebooks

12/7/21 - Team Meeting

Cora Williams - Dec 09, 2021, 10:12 AM CST

Title: Team Meeting

Date: Dec. 7, 2021

Content by: Cora Williams

Present: Karina Buttram, Josephine Hall, Georgia Hancock, Adrienne Simpson, Cora Williams

Goals:

- Review final poster

Content:

- Reviewed final poster
- Checked that all of the poster requirements were met
- Submitted the poster for printing

Conclusions/action items:

- Pick up printed poster
- Begin final report
- Continue updating lab notebooks

12/12/21 - Team Meeting

Cora Williams - Dec 12, 2021, 5:28 PM CST

Title: Team Meeting

Date: Dec. 12, 2021

Content by: Cora Williams

Present: Karina Buttram, Josephine Hall, Georgia Hancock, Adrienne Simpson, Cora Williams

Goals:

- Work on final report

Content:

- Worked on final report
- Assigned work to team members

Conclusions/action items:

- Finish final report
- Email advisor questions
- Ensure lab notebooks are up-to-date

12/14/21 - Team Meeting

Cora Williams - Dec 14, 2021, 5:34 PM CST

Title: Team Meeting

Date: Dec. 14, 2021

Content by: Cora Williams

Present: Karina Buttram, Josephine Hall, Georgia Hancock, Adrienne Simpson, Cora Williams

Goals:

- Revise final report
- Review lab notebook
- Complete client evaluation

Content:

- Revised final report
- Reviewed lab notebook
- Downloaded final lab notebook
- Completed client evaluation

Conclusions/action items:

- Submit final report
- Submit lab notebook
- Submit client cvaluation
- Submit individual and peer evaluations

9/22/21 - Initial Client Meeting

Josephine HALL (jrhall3@wisc.edu) - Oct 19, 2021, 5:46 PM CDT

Title: Initial Client Meeting

Date: Sept. 22, 2021

Content by: Cora Williams

Present: Mira Baichoo, Josephine Hall, Georgia Hancock

Goals:

- Answer general questions for project specifications

Content:

1. What is our budget?
    a. Spectrum photometer
        i. Concentration threshold
    b. Electric components
    c. 3.00 per test
2. Are you providing us with funding/ should we purchase everything on our own and reimburse after?
    a. No funding
3. What research have you and/or your team already done for this project?
    a. She did minor research on the topic and recommended two biomarkers
4. What are the expectations for this project?
    a.
5. Where would our testing device be used most?
    a. Rural Areas
6. General Information about project
    a. Design a device that is more comfortable to use than women
    b. Looking for the design to be closer to a pregnancy test.
7. Material requirements?
    a. Biocompatible
        i. No infection or inflammation
        ii. Easy to hold
        iii. Not toxic to user
        iv. Not biodegradable
8. Cultural considerations to be aware of for devices?
    a. Uncomfortable and women would go to church instead of a doctor
    b. Taboo talking about women's health in Ethiopia
        i. Want the method to be discrete
9. Other options available
    a. Blood sample
    b. Swab
    c. Saliva
10. Ideal client to use product
    a. Sexually active women in general
        i. In rural area
            1. No doctors in their area
11. Urine testing, is this the only option to be considered?
    a. No, could use blood
12. What are some current methods being used in Ethiopia today?

    a. PapSmear

13. What is the end goal product? - Design, physical device?
14. The two biomarkers suggested, have other markers been tried? Why were these two specifically selected?
15. Decision support

Conclusions/action items:

- Continue researching and updating lab notebooks
- Write Progress Report #2
- Write Product Design Specifications
- Continue meeting with group, client, and advisor

11/29/21 - Client Meeting 2

Cora Williams - Nov 29, 2021, 1:14 PM CST

Title: Client Meeting

Date: Nov. 29, 2021

Content by: Cora Williams

Present: Karina Buttram, Josephine Hall, Georgia Hancock, Adrienne Simpson, Cora Williams

Goals:

- Update client on project progress
- Listen to client suggestions about project

Content:

- Discussed prototype progress
- Discussed research progress
- Answered client questions
- Client was very impressed with our progress

Conclusions/action items:

- Continue research
- Continue prototype construction
- Begin final presentation
- Begin final report
- Send client preliminary presentation video in a different format
- Continue updating lab notebooks

9/10/21 - Advisor Meeting Week 1

Josephine HALL (jrhall3@wisc.edu) - Oct 19, 2021, 5:46 PM CDT

Title: Advisor Meeting Week 1

Date: Sept. 10, 2021

Content by: Cora Williams

Present: BME design team except for Cora

Goals:

- Get to know team members and advisor

Content:

- Advisor introduction
- Team member introductions

Conclusions/action items:

- Begin basic research
- Schedule meeting with client and team
- Update lab notebooks

9/17/21 - Advisor Meeting Week 2

Cora Williams - Sep 25, 2021, 8:15 PM CDT

Title: Advisor Meeting Week 2

Date: Sept. 17, 2021

Content by: Cora Williams

Present: Mira Baichoo, Karina Buttram, Adrienne Simpson, Cora Williams

Goals:

- Update advisor on project progress

Content:

- Meeting now from 12:30-1:00
    - Use the Zoom link from the Canvas page
- Research the specific cervical cancer marker
    - This needs to be done first before any other research
    - Link everything in the Drive
- Get access to the LabArchives
    - Once this is done we need to update it with all the information from the Drive
        - Including these meeting notes and the meeting from Tuesday Sept. 14th

Conclusions/action items:

- Meet with client
- Continue basic research
- Continue updating lab notebooks

9/24/21 - Advisor Meeting Week 3

Cora Williams - Oct 01, 2021, 1:48 PM CDT

Title: Advisor Meeting Week 3

Date: Sept. 24, 2021

Content by: Cora Williams

Present: Mira Baichoo, Karina Buttram, Josephine Hall, Georgia Hancock, Adrienne Simpson, Cora Williams

Goals:

- Update advisor on project progress
- Determine how we should fund project

Content:

- Updated advisor on project progress
    - Asked how we should fund our project, as the client was unaware of the financial commitment
    - Discussed what are realistic expectations for what we can accomplish in a semester, as we feel the client's expectations are unreasonable for a semester
    - Informed advisor of PDS and progress report status

Conclusions/action items:

- Continue research
- Begin design matrix
- Determine testing method
- Submit progress report to advisor, client, and website
- Continue updating lab notebooks

10/1/21 - Advisor Meeting Week 4

Cora Williams - Oct 01, 2021, 1:48 PM CDT

Title: Advisor Meeting Week 4

Date: Oct. 1, 2021

Content by: Cora Williams

Present: Karina Buttram, Josephine Hall, Georgia Hancock, Adrienne Simpson, Cora Williams

Goals:

- Update advisor on project progress
- Determine best bodily fluid to use for testing

Content:

- Updated advisor on project progress
    - Discussed our sample medium design matrix
    - Discussed what sample medium we should use (saliva or urine)
    - Discussed how we are going to fund our project
    - Discussed what needs to be accomplished for next week
    - Set up advisor meeting for next week

Conclusions/action items:

- Continue research
- Begin second design matrix
- Determine budget and submit funding proposal
- Continue updating lab notebooks

10/8/21 - Advisor Meeting Week 5

Cora Williams - Oct 08, 2021, 1:33 PM CDT

Title: Advisor Meeting Week 5

Date: Oct. 8, 2021

Content by: Cora Williams

Present: Mira Baichoo, Karina Buttram, Josephine Hall, Adrienne Simpson, Cora Williams

Goals:

- Update advisor on project progress

Content:

- Updated advisor on project progress
    - Discussed our testing device design matrix
    - Discussed proposed budget and funding proposal
        - We are running into issues creating a budget because we can't find any blank tests strips
    - Discussed what we are going to be looking for with our test (antibodies, proteins, or other biomarkers)
    - Discussed additional research
        - Determine what HPV biomarkers we want to look for
        - Consider using blood if we can't find a way to use urine
        - Antibody concentration differences between vaccinated women and infected women
        - Sequence on a chip to bind to the exosome sequence for HPV infection
            - After the chip binds to the exosome sequence, it will change color
            - Will probably be more expense
    - Discussed what needs to be accomplished for next week
        - Send our advisor our slides and/or progress report summary
    - Preliminary design presentation next week

Conclusions/action items:

- Continue research
- Finalize budget and submit funding proposal
- Draft preliminary design presentation
- Continue updating lab notebooks

10/18/21 - Advisor Meeting 6

Cora Williams - Oct 18, 2021, 2:08 PM CDT

Title: Team Meeting 8

Date: Oct. 18, 2021

Content by: Cora Williams

Present: Mira Baichoo, Karina Buttram, Josephine Hall, Georgia Hancock, Adrienne Simpson, Cora Williams

Goals:

- Discuss our project concerns with Dr. P
- Determine group member tasks for upcoming week

Content:

- Discussion with Dr. P about project concerns
    - Informed Dr. P of project progress thus far
    - Discussed concerns about client expectations and communication difficulties
        - Dr. P said to prioritize our learning and try to incorporate as much of the client's requirements as possible
    - Discussed concerns about reasonable project end goals
        - Client and advisor want a fully functioning test
        - The team does not believe that this is feasible given time constraints
        - Dr. P said that there will be no issue if we don't have a fully functioning test by the end of the semester
        - We just need to try to get as far as possible
    - Discussed concerns about advisor meetings
        - The team feels that we don't make progress in advisor meetings
        - The team also feels like we discuss the same things every week and that the team and our advisor are never on the same page
        - After watching preliminary presentations last week, the team felt like our advisor might be confusing us with the other project he advises
        - Dr. P said that he will remind all advisors what preliminary deliverables should look like
        - He also said that he will talk to our advisor
    - Dr. P gave the team lots of additional resources that may be useful
        - Two different potential contacts
        - Project information from two previous projects
- Distributed work to all group member
    - Adrienne
        - Preliminary designs
        - Preliminary design evaluation
    - Cora

        - Background
    - Georgia
        - Conclusions
    - Josie
        - Results
    - Karina
        - Fabrication/Development Process
    - Mira
        - Introduction
        - Discussion

Conclusions/action items:

- Continue research
- Work on preliminary report
- Continue updating lab notebooks

10/19/21 - Advisor Meeting 7

Josephine HALL (jrhall3@wisc.edu) - Oct 19, 2021, 1:19 PM CDT

Title: Preliminary Deliverables Expectations

Date: 10/19/2021

Content by: Josephine Hall

Present: Dr. Qian

Goals: Review expectations for preliminary deliverables

Content:

Preliminary deliverables expectations:

- Theoretical testing section describing how we would test our biomarker (in a general manner)
- Results section describing the two potential biomarkers and the further research that needs to be done to choose between the two.

Next Week:

- Further research on both biomarkers
    - Determine if there is an antibody concentration difference in HPV positive and negative women regardless of vaccination status
        - If there is not a detectable difference, we will rule this biomarker out
    - Determine the stage of infection that the oncoprotein appears
        - Ensure that the protein is not present in vaccinated population
    - Research on what the protein reacts with/ binds to

Conclusions/action items: Created an outline of the expectations for preliminary deliverables and work to be done in the following week.

10/22/21 - Advisor Meeting 8

Cora Williams - Oct 22, 2021, 1:45 PM CDT

Title: Advisor Meeting 8

Date: Oct. 22, 2021

Content by: Cora Williams

Present: Mira Baichoo, Karina Buttram, Josephine Hall, Georgia Hancock, Adrienne Simpson, Cora Williams

Goals:

- Discuss our progress with advisor
- Determine group member tasks for upcoming week

Content:

- Discussed our project progress with our advisor
    - Discussed preliminary presentation grader comments
        - Drawings were too simple
            - Create Solidworks models of all designs
            - Need to add dimensions
        - Needed to elaborate on competing designs
        - Add additional details about collection methods/devices
        - Improve figure and table labels
- Distributed work to all group member
    - Cora, Josie, Karina
        - Additional research on biomarker
    - Adrienne, Georgia, Mira
        - Start fabrication

Conclusions/action items:

- Continue research
- Start fabrication
- Continue updating lab notebooks

10/29/21 - Advisor Meeting 9

Cora Williams - Oct 29, 2021, 1:32 PM CDT

Title: Advisor Meeting 9

Date: Oct. 29, 2021

Content by: Cora Williams

Present: Karina Buttram, Josephine Hall, Georgia Hancock, Adrienne Simpson, Cora Williams

Goals:

- Discuss our progress with advisor
- Determine group member tasks for upcoming week

Content:

- Discussed our project progress with our advisor
    - Discussed initial 3-D printed prototype
        - Solidworks models look significantly better
        - We ended up printing the prototype in two pieces, as it is hollow
            - It will need to be glued together then
        - Add a scale in our Solidworks model for the final report
    - Discussed research progress
        - We decided to pursue using the E6/E7 oncoproteins
        - We are also considering adding a second test strip for an antibody
        - We still need to do more research on the E6/E7 oncoproteins peptide sequence and if there is a viable antibody
            - Are there any strains of cervical cancer not related to HPV?
            - Are E6/E7 oncoproteins present in cervical cancer caused by HPV strains other than HPV 16 and HPV 18?
- Distributed work to all group member
    - Cora, Josie, Karina
        - Additional research on antibodies
        - Determine what peptide E6/E7 binds to
    - Adrienne, Georgia, Mira
        - Continue fabrication
        - Begin writing testing protocols

Conclusions/action items:

- Continue research
- Continue fabrication
- Continue updating lab notebooks

11/12/21 - Advisor Meeting 10

Cora Williams - Nov 21, 2021, 10:41 PM CST

Title: Advisor Meeting 10

Date: Nov. 12, 2021

Content by: Cora Williams

Present: Mira Biachoo, Karina Buttram, Josephine Hall, Georgia Hancock, Adrienne Simpson, Cora Williams

Goals:

- Discuss our progress with advisor
- Determine group member tasks for upcoming week

Content:

- Reported on how the BME Show and Tell went last Friday
- Discussed our project progress with our advisor
    - Discussed initial 3-D printed prototype
        - Decided that we now need two test strips instead of the three we discussed at Show and Tell
        - The "bubble" on the testing apparatus needs some revising, as it collapsed in on itself the first time it was printed
        - We also decided that we are going to print in resin from here on out, as it should result in a better print quality than PLA
    - Discussed research progress
        - We determined the peptide sequences that the E6 and E7 oncoproteins bind to
        - We still need to figure out how the color change will occur
        - We still need to figure out how the control test will work
- Distributed work to all group member
    - Cora, Josie, Karina
        - Additional research on potential control tests
        - Additional research on color changing reaction
    - Adrienne, Georgia, Mira
        - Update SolidWorks model
        - Printed updated prototype
        - Begin writing testing protocols

Conclusions/action items:

- Continue research
- Continue fabrication
- Continue updating lab notebooks

11/19/21 - Advisor Meeting 11

Cora Williams - Nov 21, 2021, 10:50 PM CST

Title: Advisor Meeting 11

Date: Nov. 19, 2021

Content by: Cora Williams

Present: Mira Baichoo, Karina Buttram, Josephine Hall, Georgia Hancock, Adrienne Simpson, Cora Williams

Goals:

- Discuss our progress with advisor
- Determine group member tasks for upcoming week

Content:

- Discussed our project progress with our advisor
    - Discussed prototype team progress
        - Ordered droppers
        - Received droppers and test strip materials
    - Discussed research progress
        - We were not able to find what pregnancy tests use to do the control test, even when looking at a patent
            - Advisor suggested looking through the patent again, as it should tell us exactly how it works
        - We are looking into using nonmetals (specifically telerium and selenium) for our control test
        - Josie foundout that a pH test won't work as a good control test when using urine because there is too much variation in normal urine
- For next week, we need to:
    - Figure out how to get the color to show
        - Potentially link the dye to the peptide sequence
    - Determine the binding affinity for E6/E7 oncoproteins to their respective peptide sequences
    - Potentially have a consultation meeting with the BioTECH team
    - Potentially talk to professors on campus who are working on similar projects
- By our next advisor meeting, our advisor wants us:
    - To know what dye we will use and how it works
    - To have the prototype printed and assembled
- Distributed work to all group member
    - Cora, Josie, Karina
        - Continue research
    - Adrienne, Georgia, Mira
        - Continue fabrication

    - Write testing protocols

Conclusions/action items:

- Continue research
- Continue fabrication
- Continue updating lab notebooks

11/22/21 - Advisor Meeting 12

Cora Williams - Dec 01, 2021, 8:04 PM CST

Title: Team Meeting 12

Date: Nov. 22, 2021

Content by: Cora Williams

Present: Mira Baichoo, Karina Buttram, Josephine Hall, Georgia Hancock, Adrienne Simpson, Cora Williams

Goals:

- Discuss our project concerns with Dr. P

Content:

- Discussion with Dr. P about project concerns
    - Informed Dr. P of project progress thus far
    - Discussed concerns about advisor expectations
        - The team feels that our advisor still has unreasonable expectations for final product
            - He wants a fully functioning thereotical test
        - The team feels that these expectations are unrealistic given the time remaining
    - Dr. P gave the team some potential solutions for our problems
        - Dyes that change color when in the presence of proteins (brown to blue)
        - Continue looking into how pregnancy tests work
            - Try looking at peer-reviewed articles
        - Potentially look into how "paper" tests work
        - Recommended we look into a certain researcher's (George Whiteside) work
            - https://gmwgroup.harvard.edu/low-cost-diagnostics-and-tools-global-health
            - He uses a wax printer to print a chemical reaction onto a test strip
            - https://gmwgroup.harvard.edu/publications/simple-telemedicine-developing-regions-camera-phones-and-paper-based
        - See if we can purchase E6/E7 oncoproteins
            - Can include in our report that it was too expense
        - Try to determine how much E6/E7 is in urine
            - Maybe look at the patents for OncoE6
        - https://www.sciencedirect.com/sdfe/pdf/download/eid/3-s2.0-B9780080970370000361/first-page-pdf
        - https://www.sciencedirect.com/topics/medicine-and-dentistry/pregnancy-test
        - https://search.library.wisc.edu/catalog/9910007221202121
        - https://www.abcam.com/hpv16-e6-hpv18-e6-antibody-c1p5-ab70.html
        - https://www.abcam.com/human-papillomavirus-16-e7-antibody-tvg-701y-ab20191.html
        - "Anti-Mouse Antibody" available in the teaching labs

    - Dr. P feels that we can use a pregnancy test control as our control test
    - Dr. P suggested that we should just use one test strip
    - Dr P. feels that it is reasonable for us to have a printed prototype and a thereotically working test strip

Conclusions/action items:

- Continue research
- Continue updating lab notebooks

12/3/21 - Advisor Meeting 13

Cora Williams - Dec 03, 2021, 1:20 PM CST

Title: Team Meeting 13

Date: Dec. 3, 2021

Content by: Cora Williams

Present: Karina Buttram, Josephine Hall, Georgia Hancock, Adrienne Simpson, Cora Williams

Goals:

- Discuss our project progress with our advisor

Content:

- Discussion with our advisor about the progress we made this week
    - Presented our printed prototype
    - Discussed research progress
        - We determined the antibodies, test strips, and dyes that we need
        - We will be closely modeling a pregnancy test
- Discussion with our advisor about our plans for the poster presentation
    - Discussed what we are bringing to the presentation
    - Discussed what will be on our poster
        - Include lots of diagrams and fewer words
        - Put future testing in future work section
        - Could include other preliminary designs in testing section as it was kinda a "test"
- Advisor was pleased with the work we put into the project and the information we provided

Conclusions/action items:

- Continue final presentation
- Continue final report
- Continue updating lab notebooks

10/4/21 - Design Matrix - Sample Type

Georgia Hancock - Oct 04, 2021, 4:50 PM CDT

Design Categories	#1 Blood		#2 Saliva		#3 Urine	
Prior Detection (30)	3/5	18	3/5	18	4/5	24
Ease of Obtaining Usable Sample (25)	**4/5**	**20**	3/5	15	**4/5**	**20**
Comfort (20)	2/5	8	**5/5**	**20**	4/5	16
Ease of Collection (15)	2/5	6	**5/5**	**15**	4/5	12
Storage Requirements (10)	2/5	4	**5/5**	**10**	4/5	8
Total (100)	56		78		**80**	

Prior Detection:

The most important factor in which substance we would choose to test is how sure we could be that it would be able to accurately and efficiently produce a result for HPV. Since the accuracy will depend on our specific testing method and studies on detecting HPV in these substances vary in percent accuracy, the number we chose to base our decision on was how much the sample type had been previously used by other researchers.

Ease of Collection:

Each sample that can possibly be tested requires different methods of collection. The collection method should be the one that is easiest to obtain a sample from and require no prior knowledge of medical procedures.

Comfort:

Patient comfort was an important consideration for this decision. Each sample medium collection process results in slightly different amounts of pain for the patient. The pain level of the patient should be minimal to non-existent while collecting the sample.

Ease of Obtaining Usable Sample: Different sample mediums can require slight variations in collection in order to obtain a usable sample. The sample medium should not require prior planning or complex techniques for the collected sample to be usable.

Design_Martix.docx(8.5 KB) - download

10/5/21 - Design Matrix - Collection Device

Georgia Hancock - Oct 05, 2021, 1:13 PM CDT

Design Categories	#1 Strip Dip 		#2 Bean in a Bed 		#3 Sleeve 	
Ease of Use (30)	3/5	18	4/5	24	**5/5**	**30**
Cost (25)	**5/5**	**25**	3/5	15	2/5	10
Ease of Fabrication (20)	**5/5**	**20**	4/5	16	2/5	8
Sample Containment (15)	3/5	9	**5/5**	**15**	2/5	6
Efficiency (10)	4/5	10	**5/5**	**10**	3/5	6
Total (100)	**82**		10		60	

Ease of Use: - Josie

The criteria with the highest weight and importance is the ease of use. The user must be able to understand how the device is used and easily perform the desired tasks to collect a sample for testing.

Cost: - Mira

Each sample that can possibly be tested requires different methods of collection. The collection method should be the one that is easiest to obtain a sample from and require no prior knowledge of medical procedures.

Ease of Fabrication: - Adrienne

Sample Containment: Cora

Design_Martix_Collection_Device_.pdf(1 MB) - download

10/19/2021 - Preliminary Solid Works

Josephine HALL (jrhall3@wisc.edu) - Oct 19, 2021, 5:54 PM CDT

Title: Preliminary Solid Works Design

Date: 10/19/2021

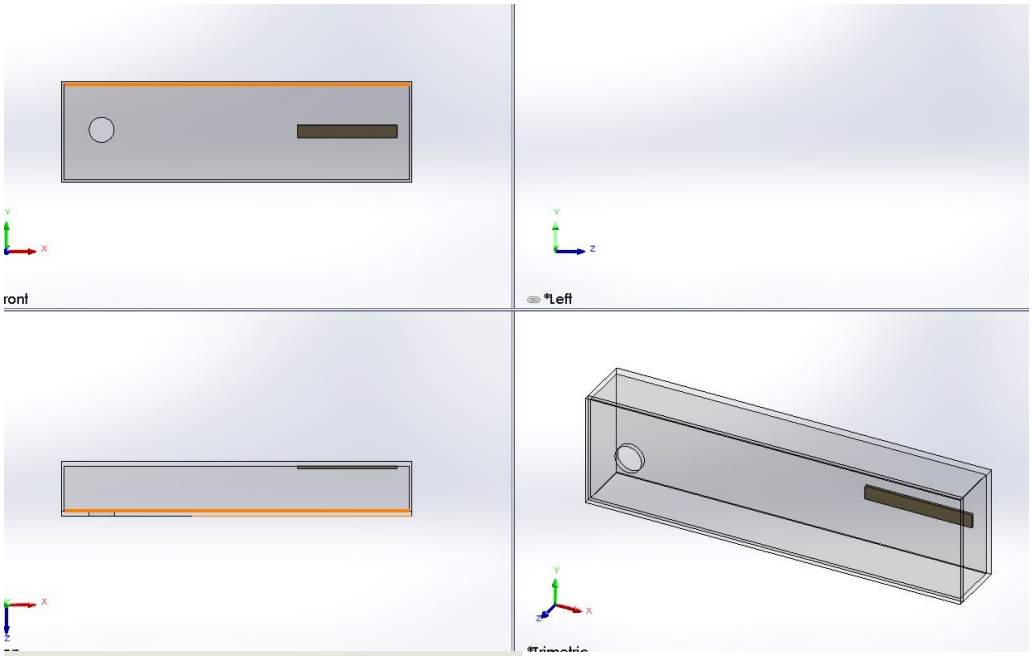
Content by: Josie Hall

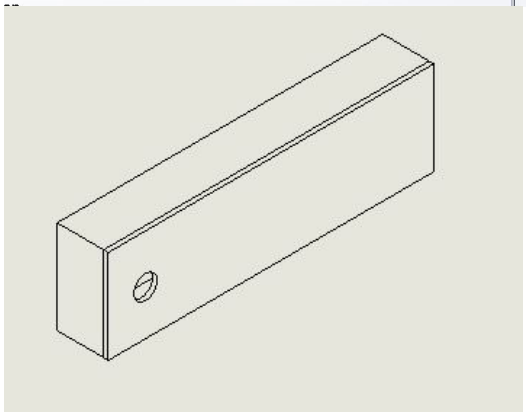
Present: Josie Hall

Goals:

To create a preliminary solid works model on selected collection device for fabrication.

Content:





Conclusions/action items:

This is the SolidWorks model of the preliminary design for our collection device. The device has not yet been properly dimensioned.

10/27/2021 - Research Team Findings

Josephine HALL (jrhall3@wisc.edu) - Oct 27, 2021, 7:15 PM CDT

Title: Research Team findings

Date: 10-/27/2021

Content by: Josephine Hall

Present: Josephine, Karina, Cora

Goals: Document discussion and findings

Content:

- Cora found a cervical screening test using e6 oncoprotein (using lab techniques)
- Karina and Josephine found levels IgG and IgA but these are mostly associated with illness and not HPV specific - these will not be good options moving forward
- Discussed testing for two potential markers
- Karina found that E6 can be detected up to 20-30 years prior to infection becoming cancerous
- E6 and E7 are needed for HPV to become cancerous
    - would not show up in vaccinated and uninfected women

Conclusions/action items: We're going to likely use E6 and E7 to test, potential to add another biomarker (split the testing apparatus down middle and use two strips). Potential to use monoclonal antibodies for HPV 16 and HPV 18.

10/26/21 - Initial Prototype Model

Georgia Hancock - Oct 28, 2021, 10:05 AM CDT

Title: Initial Prototype

Date: 10/26/21

Content by: Georgia Hancock

Present: Georgia Hancock, Mira Baichoo, Adrienne Simpson

Goals: Finalize Solidworks Model

Content:

-Created a new Solidworks model from scratch to account for the hollow inside

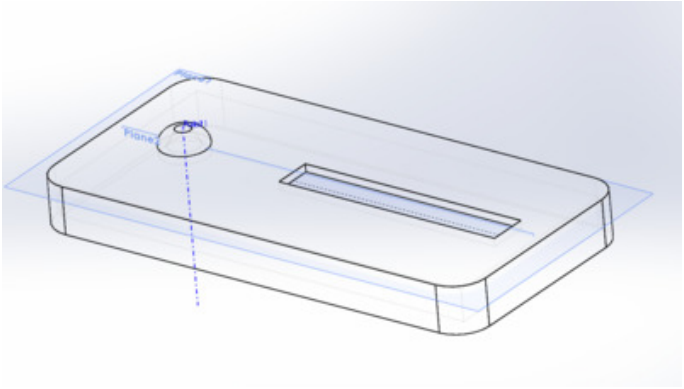
-Added holes for viewing and sample deposit

-Decided to make the sample deposit hole a "bubble" for maximum ease of use by allowing the user to insert the dropper into the bubble and have the sample reverse funnel onto the absorbent pad

Conclusions/action items:

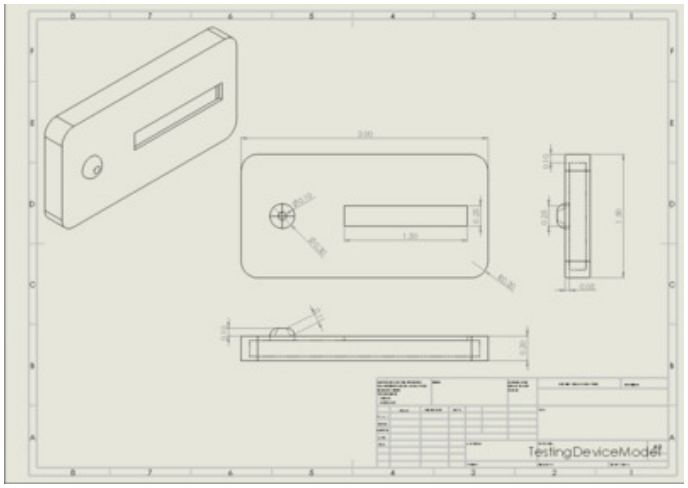
-3D print at Makerspace!

Georgia Hancock - Oct 28, 2021, 10:05 AM CDT



TestingDeviceComplete.PNG(150.9 KB) - download Completed testing device model

Georgia Hancock - Oct 28, 2021, 10:21 AM CDT



TestingDeviceDrawing.PNG(28.2 KB) - download Model Drawing with dimensions (in)

10/28/21 - Cup Design 1 SolidWorks File

MIRA BAICHOO - Oct 28, 2021, 10:05 AM CDT

Cup_Design_1.SLDPRT(128.5 KB) - download

MIRA BAICHOO - Oct 28, 2021, 10:05 AM CDT

Title: SolidWorks Design 1

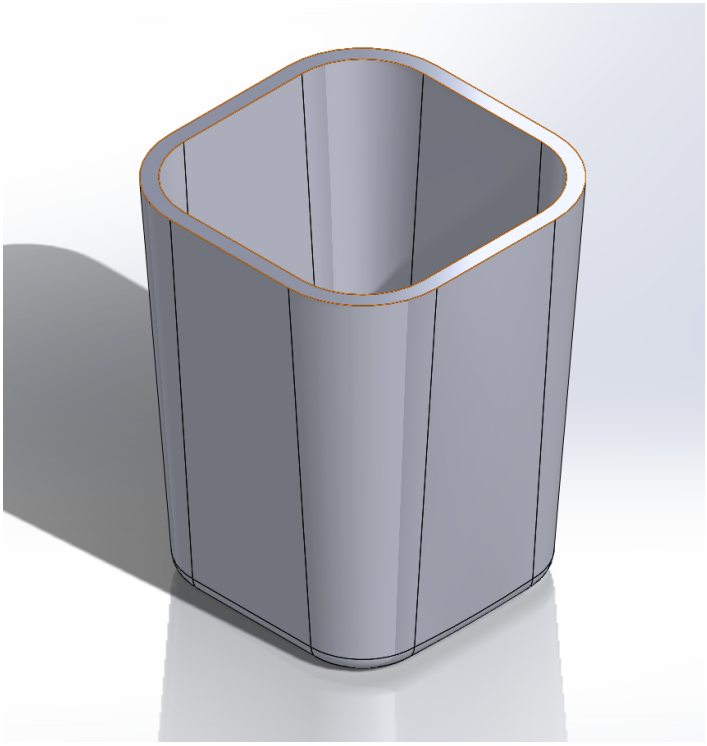
Date: 10/28/2021

Content by: Mira Baichoo

Present: N/A

Goals: Get a SolidWorks prototype for design one

Content:



Conclusions/action items:

Have the rest of the team look at the design and confirm dimensions.

11/3/21 - Funnel Design

ADRIENNE SIMPSON - Nov 03, 2021, 7:14 PM CDT

Title: Funnel Device Autocad

Date: 11/3/21

Content by: Adrienne Simpson

Present: Adrienne Simpson

Goals: Make a 3D model of funnel device

Content: Funnel Device Drawing below

Conclusions/action items:

Have teammates check over design.

ADRIENNE SIMPSON - Nov 03, 2021, 7:13 PM CDT

Funnel_Device_Drawing-Model.pdf(289.4 KB) - download

11/5/21 - Show and Tell Feedback

KARINA BUTTRAM - Nov 05, 2021, 1:42 PM CDT

Title: Show and Tell Feedback

Date: 11/5/2021

Content by: Team

Present: Team

Goals: determine any changes or alteration we need to make to our design

Content:

- three holes vs. one hole for the urine sample on device, three is easier to fabricate but one is more user friendly

- definitely changing printing material to resin

- color change : color blind individuals? research types of color blindness most common in women

- creating a lip inside the device to hold the test strips in place

- redesigning device for three test strips

- make it clear that if any strip shows positive result it is a concern and should be tested more using lab techniques

Conclusions/action items: We are going to redesign the device to have three test strips and determine if we should have three bubbles of one that funnels to each test strip on the device. We will further research color changing reactants and chose a color that will be seen by color-blind individuals.

11/8/21 - Biotechnology Center Information

KARINA BUTTRAM - Nov 08, 2021, 1:03 PM CST

Title: Biotechnology Center Information

Date: 11/8/2021

Content by: Karina Buttram

Present: Karina Buttram

Goals: Determine if the biotechnology center will be able to help us replicate peptide sequences

Content: https://biotech.wisc.edu/uwbc-services/

Genome Assembly: they have replicated genomes of several organisms, would work with us to determine a plan of how to replicate the sequence, trial and error process of isolating a specific sequence, create an assembly report

Conclusions/action items: I am not certain that the biotech center would be able to help us replicate the peptide sequences we need, but it would be worth contacting them to be certain.

Expenses Table

Title: Expenses Table

Date: 10/1/2021

Content by: Karina Buttram

Present: Karina Buttram

Goals: list all of our purchases and materials used

Content:

Item	Description	Manufacturer	Part Number	Date	QTY	Cost Each	Total	
Mini Pipettes	small pippettes used to drop liquid into a small openning	V Cool Livat	shvk-science eye droppers	11/10/21	100	$0.05	$5.19	https://www.amazon.com/dp/B07MSNQYTV/ref=syn_sd_onsite_desktop_114?psc=1&pd_rd_plhdr=t&spLa=ZW5jcnlwdGVkUXVhbGlmaWVyPUExU1M5UjdUVVVXMU1EJmVuY3J5cHF
TOUGH resin filament	any filament used while 3D printing	MakerSpace		11/3/21 12/1/21	2 prototypes	$1.06 $7.30	$8.36	
Super Glue	glue two sides of the prototype together (already had this)	Super Glue	SGCSGH2		1	$0.00	$0.00	https://www.amazon.com/Super-Glue-SGH2-48-Single/dp/B00009V3VE/ref=asc_df_B00009V3VE/?tag=h
							Total: $13.55	

Conclusions/action items: These are our expenses for this semester.

11/22/2021 - E6 and E7 Antibody and Protein Costs

Josephine HALL (jrhall3@wisc.edu) - Nov 22, 2021, 4:06 PM CST

Title: E6 and E7 Antibody Costs

Date: 11/22/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Document the costs of E6 and E7 antibody

Content:

links to website: Anti-HPV16 E6 + HPV18 E6 antibody [C1P5] (ab70) | Abcam , Anti-Human Papillomavirus 16 (E7) antibody [TVG 701Y] (ab20191) | Abcam , Anti-HPV18 E7 antibody [8E2] (ab100953) | Abcam , Recombinant HPV16 E6 protein (His tag) (ab226447) | Abcam , Recombinant Human papillomavirus HPV18 E7 protein (His tag) (ab236931) | Abcam , Recombinant Human Papillomavirus 16 (E7) protein (His tag) (ab237790) | Abcam

- Anti-HPV16 E6 + HPV18 E6 antibody - $445 per 100 µg

- Anti-HPV18 E7 antibody - $480 per 100 µl
- Anti-Human Papillomavirus 16 (E7) antibody - $445 per 100 µg
- Recombinant HPV16 E6 protein - $510 per 100 µg
- Recombinant Human papillomavirus HPV18 E7 protein - $950 per 100 µg
- Recombinant Human Papillomavirus 16 (E7) protein - $590 per 100 µg

Conclusions/action items: To begin creating our testing device, it would cost us $1370 to acquire all desired antibodies and an additional $2050 to acquire the E6/E7 proteins to test our device.

Blue Latex Particles

KARINA BUTTRAM - Nov 30, 2021, 3:41 PM CST

Title: Colored Polystyrene Particles

Date: 11/30/2021

Content by: Karina Buttram

Present: Karina Buttram

Goals: have an idea for the cost of blue latex particles

Content: https://www.magsphere.com/Products/Colored-Polystyrene-Particles/colored-polystyrene-particles.html

$154 for 5ml of 0.08 μm diameter particles

Conclusions/action items: It will cost us $154 for the blue latex particle that we will use to coat the antibodies to use for the control line.

Expected Expenses Table

Title: Expenses Table

Date: 10/1/2021

Content by: Karina Buttram

Present: Karina Buttram

Goals: list all of our purchases and materials used, and all of the materials we would need to purchase in the future

Content:

Item	Description	Manufacturer	Part Number	Date	QTY	Cost Each	Total	
Mini Pipettes	small pippettes used to drop liquid into a small openning	V Cool Livat	shvk-science eye droppers	11/10/21	100	$0.05	$5.00	https://www.amazon.com/dp/B07MSNQYTV/ref=syn_sd_onsite_desktop_114?psc=1&pd_rd_plhdr=t&spLa=ZW5jcnlwdGVkUXVhbGlmaWVyPUExU1M5UjdUVVVXMU1EJm
TOUGH resin filament	any filament used while 3D printing	MakerSpace		11/3/21 12/1/21	2 porotypes	$1.06 $7.30	$8.36	
Clear cups	5.0 fl oz. clear, plastic cups	Prestee	B0757YV1W1		100	$0.16	$16.00	https://www.amazon.com/Plastic-Disposable-Cocktail-Drinking-Tumblers/dp/B0757YV1W1/ref=
Super Glue	glue two sides of the prototype together	Super Glue	SGCSGH2		1	$2.79	$2.79	https://www.amazon.com/Super-Glue-SGH2-48-Single/dp/B00009V3VE/ref=asc_df_B00009V3
Anti-HPV 16 + 18 E6 antibody		abcam			100 µg	$4.45 per µg	$445.00	https://www.abcam.com/hpv16-e6-hpv18-e6-antibody-c1p5-ab70.html
Anti-HPV 16 E7 antibody		abcam			100 µg	$4.45 per µg	$445.00	https://www.abcam.com/human-papillomavirus-16-e7-antibody-tvg-701y-ab20191.html
Anti-HPV 18 E7 antibody		abcam			100 µl	$4.80 per µl	$480.00	https://www.abcam.com/hpv18-e7-antibody-8e2-ab100953.html
Recombinant HPV16 E6 protein		abcam			100 µg	$5.10 per µg	$510.00	https://www.abcam.com/recombinant-hpv16-e6-protein-his-tag-ab226447.html
Recombinant Human papillomavirus HPV18 E7 protein		abcam			100 µg	$9.50 per µg	$950.00	https://www.abcam.com/recombinant-human-papillomavirus-hpv18-e7-protein-his-tag-ab23693
Recombinant Human Papillomavirus 16 (E7) protein		abcam			100 µg	$5.90 per µg	$590.00	https://www.abcam.com/recombinant-human-papillomavirus-16-e7-protein-his-tag-ab237790.h
Goat Anti-Rabbit IgG H&L		abcam			500 µg	$0.28 per µg	$140.00	https://www.abcam.com/goat-rabbit-igg-hl-alexa-fluor-488-ab150077.html
Recombinant Anti-Rabbit IgG antibody		abcam			100 µl	$5.20 per µl	$520.00	https://www.abcam.com/alexa-fluor-488-rabbit-igg-antibody-sp137-ab270142.html
Universal Lateral Flow Assay Kit		abcam			100 tests	$16.60 per test	$1660.00	https://www.abcam.com/universal-lateral-flow-assay-kit-ab270537.html
Blue Polystyrene Latex Particles	0.08 µm diameter particles	Magsphere	PSB080NM		5 mL	$30.80 per L	$154.00	https://www.magsphere.com/Products/Colored-Polystyrene-Particles/colored-polystyrene-partic
Anti-p53 Antibody		abcam			100 µg	$4.60 per µg	$460.00	https://www.abcam.com/p53-antibody-pab-240-ab26.html

Item	Description	Manufacturer	Part Number	Date	QTY	Cost Each	Total	
Recombinant anti-pRb antibody		abcam			100 µl	$5.30 per µl	$530.00	https://www.abcam.com/rb-antibody-epr17512-ab181616.html
Recombinant anti-UBE3A antibody		abcam			100 µl	$4.80 per µl	$480.00	https://www.abcam.com/ube3a-antibody-epr23077-14-ab272168.html
							Total: $7396.15	

Conclusions/action items: These are our future expenses if we were to continue this project and fulfill the future tests.

10/28/21 - 3D Printing Session - Initial Prototype

Georgia Hancock - Oct 28, 2021, 10:29 AM CDT

Title: 3D Printing Session - Initial Prototype

Date: 10/28/21

Content by: Georgia Hancock

Present: Georgia Hancock, Mira Baichoo

Goals: Choose material and begin 3D print process

Content:

-Realized it would be impossible to 3D print a hollow device

-Improvised by separating device into "lid" and base to print separately and then glue together

-Chose to print from plastic PLA material for lowest cost. Resin was an option for increased strength but the makerspace staff recommended the PLA since our device will not need to withstand any heavy loads or forces

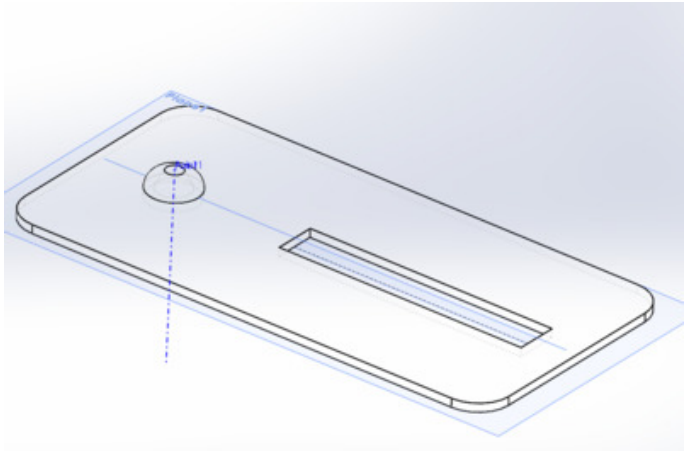
-Created two separate Solidworks models for the lid and base to print separately

Conclusions/action items:

-Pick up finished 3D print jobs tomorrow

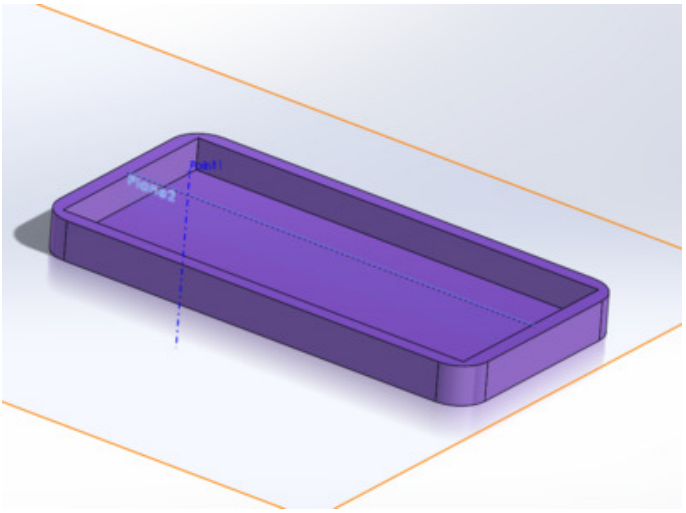
-Assemble next week

Georgia Hancock - Oct 28, 2021, 10:30 AM CDT



TestingDeviceTop.PNG(128.9 KB) - download Lid model

Georgia Hancock - Oct 28, 2021, 10:30 AM CDT



testingDeviceBottom.PNG(131.8 KB) - download Base model

Georgia Hancock - Dec 14, 2021, 5:32 PM CST

IMG_6153_2.HEIC(1.7 MB) - download

Georgia Hancock - Dec 14, 2021, 5:32 PM CST

IMG_6154_2.HEIC(1.3 MB) - download

Georgia Hancock - Dec 14, 2021, 5:32 PM CST

IMG_6159_2.HEIC(1.4 MB) - download

11/3/21 - Initial Prototype Print

Georgia Hancock - Nov 03, 2021, 7:19 PM CDT

Title: Initial Prototype Print

Date: 11/3/21

Content by: Georgia Hancock

Present: Mira Baichoo, Adrienne Simpson

Goals: Establish next steps in our prototyping process based on initial print

Content:

-Initial print has some flaws

-Bubble did not print correctly and broke off, needs to be resized

-Hole sizes look appropriate

-Depth looks good, lid could be slightly thicker to make it easier to handle

-Gorilla glue sealed it well, will confirm with water test on final

Conclusions/action items:

-Create new prototype and print once we confirm number of test strips with research team

Georgia Hancock - Nov 03, 2021, 7:26 PM CDT

IMG_6159_2.HEIC(1.4 MB) - download

Georgia Hancock - Nov 03, 2021, 7:27 PM CDT

IMG_6158_2.HEIC(1.4 MB) - download

Georgia Hancock - Nov 03, 2021, 7:27 PM CDT

IMG_6155_2.HEIC(1.2 MB) - download

Georgia Hancock - Nov 03, 2021, 7:27 PM CDT

IMG_6154_2.HEIC(1.3 MB) - download

Georgia Hancock - Nov 03, 2021, 7:27 PM CDT

IMG_6153_2.HEIC(1.7 MB) - download

Georgia Hancock - Nov 03, 2021, 7:27 PM CDT

IMG_6152_2.HEIC(1.4 MB) - download

Georgia Hancock - Nov 03, 2021, 7:27 PM CDT

IMG_6150_2.HEIC(1.9 MB) - download

11/10/21 - Prototype Model Update

Georgia Hancock - Nov 10, 2021, 6:53 PM CST

Title: Prototype Model Update

Date: 11/10/21

Content by: Georgia Hancock, Adrienne Simpson

Present: Georgia Hancock, Adrienne Simpson

Goals: To create an updated prototype drawing

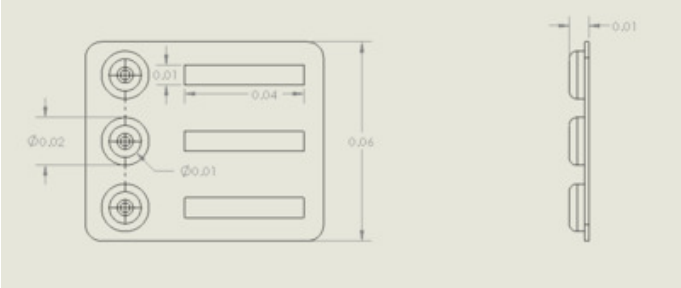
Content:

-Created updated drawing with bigger dropper insertion bubbles to ensure they don't collapse while printing

-Increased width and 3 test strip sections with 3 separate dropper bubbles

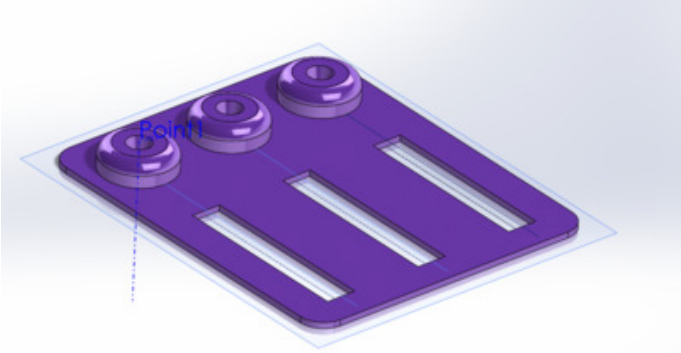
Conclusions/action items: Need to set up a meeting with Makerspace to print updated prototype

Georgia Hancock - Nov 10, 2021, 6:53 PM CST



Lid2.0_Drawing.PNG(10.9 KB) - download

Georgia Hancock - Nov 10, 2021, 6:53 PM CST



Lid_2.0.PNG(254.3 KB) - download

11/29/21 - Prototype Model Update 2

Georgia Hancock - Dec 01, 2021, 6:52 PM CST

Title: Prototype Design Update

Date: 11/29/21

Content by: Georgia Hancock

Present: Georgia Hancock

Goals: Create a new model for only 1 test strip

Content:

After some guidance from Dr. Puccinelli, we have decided to go back to a single test strip model, so the Solidworks needed to be updated to have only 1 row while maintaining the new design of the more rigid deposit bubble.

Conclusions/action items:

Print new prototype

Georgia Hancock - Dec 14, 2021, 5:34 PM CST

Screen_Shot_2021-12-14_at_5.33.55_PM.png(342.3 KB) - download

12/14/21 - Mechanical Testing Protocols

ADRIENNE SIMPSON - Dec 14, 2021, 5:45 PM CST

Title: Mechanical Testing Protocols

Date: 12/14/21

Content by: Adrienne

Present: Georgia, Adrienne, Mira

Goals: To specify the testing protocols used

Content:

Durability Testing

To test for potential mechanical failures and prototype durability, the device casing was handled with slight pressure to simulate being used. It was then observed to ensure all the components held up and stayed together during use.

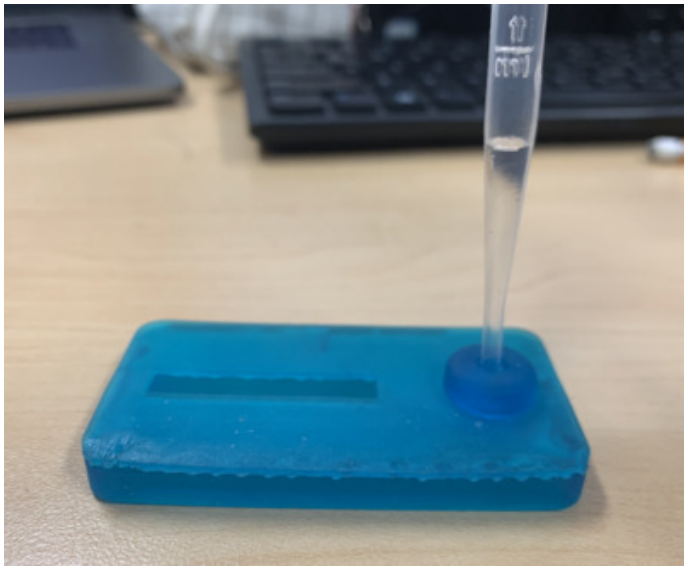
Leakage Testing

To test for potential device leakage, water was dropped into the device casing using a pipette to simulate a urine sample. A 5 mL water sample was tested in the device to simulate the approximate sample size form the user. A 3 mL water sample was also tested in the device to simulate user error in the case that the user puts too much liquid into the test. It was then observed to ensure there was no leakage after two minutes. The test strip was not needed in the device to test for potential leakage.

Conclusions/action items:

After testing the results need to be recorded.

Georgia Hancock - Dec 14, 2021, 5:34 PM CST



Screen_Shot_2021-12-07_at_5.26.14_PM.png(3.4 MB) - download Leakage testing

12/14/21 - Mechanical Testing Results

ADRIENNE SIMPSON - Dec 14, 2021, 5:44 PM CST

Title: Mechanical Testing Results

Date: 12/14/21

Content by: Adrienne

Present: Georgia, Adrienne, Mira

Goals: To record the results from the mechanical tests

Content:

Durability Test

For the durability test, the original prototype made of PLA with a smaller insertion bubble didn't print correctly and the bubble collapsed upon use of the device. The prototype was updated so that the insertion bubble is bigger and more sturdy and the new material was "TOUGH" resin. Upon testing of the updated prototype, the bubble held up against usage and the "TOUGH" resin prototype printed a lot better than the original prototype.

Leakage Test

For the leakage test, after two minutes of observation, neither the .5 mL nor the 3 mL sample leaked out of the device. This test was only run on the update prototype and it was concluded that this prototype passed the leakage test.

Conclusions/action items:

Mechanical tests were run and the results showed what needed to be improved upon in our device casing. The prototype bubble was made bigger and the material was switched for better printing.

12/05/21 - Mechanical Testing

Georgia Hancock - Dec 14, 2021, 5:41 PM CST

Title: Mechanical testing session

Date: 12/5/21

Content by: Georgia Hancock

Present: Georgia Hancock

Goals: Test our final prototype to ensure durability and anti-leakage

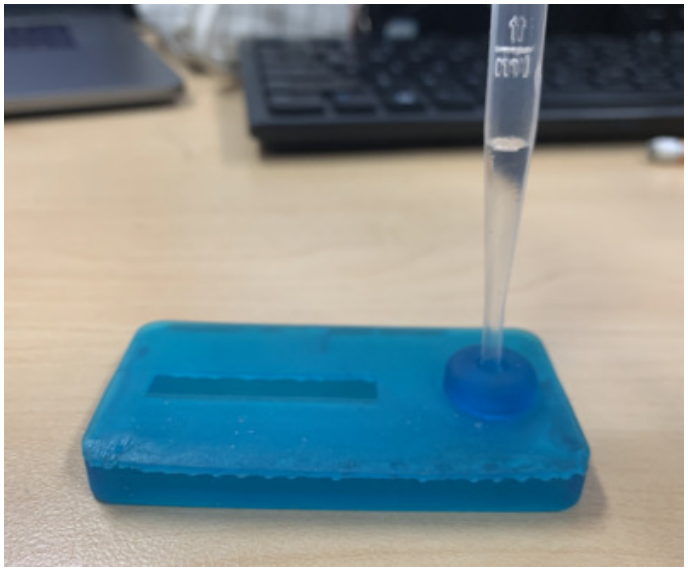
Content:

-After following protocol for leakage testing, prototype passed both 5mL and 3mL scenarios

-Prototype held up to durability standards

Conclusions/action items:

Georgia Hancock - Dec 14, 2021, 5:41 PM CST



Screen_Shot_2021-12-07_at_5.26.14_PM.png(3.4 MB) - download Leakage test

11/15/21 - Mechanical Failure of Initial Prototype

Georgia Hancock - Dec 14, 2021, 5:46 PM CST

Title: Mechanical failure of initial prototype

Date: 10/29/21

Content by: Georgia Hancock

Present: Georgia Hancock, Mira Baichoo, Adrienne Simpson

Goals: Determine future improvements for prototype given failure

Content:

After picking up initial prototype print and assembling top and bottom piece and removing excess support material, the sample insertion "bubble" on the device failed and collapsed, leaving only our base hole. We decided we will need to reconstruct the hole in Solidworks as well as explore potential tougher print materials such as resin as opposed to PLA. This has made us realize that we should establish a basic testing protocol to ensure our device meets usability standards.

Conclusions/action items:

Redesign prototype

Establish testing protocols

10/19/2021 - Design Matrix 1

Cora Williams - Oct 19, 2021, 5:58 PM CDT

Title: Design Matrix 1

Date: Oct. 19, 2021

Content by: Cora Williams

Present: BME Design Team

Goals:

- Determine what bodily fluid to use to test for HPV

Content:

- See Design Matrix file below

Conclusions/action items:

We decided to use urine as our testing medium. Now that we have decided on a testing medium, we need to design a collection and testing device.

Cora Williams - Oct 19, 2021, 5:56 PM CDT

Design Categories	#1 Blood		#2 Saliva		#3 Urine	
Prior Detection (30)	3/5	18	3/5	18	4/5	24
Ease of Obtaining Usable Sample (25)	4/5	20	3/5	15	4/5	20
Comfort (20)	2/5	8	5/5	20	4/5	16
Ease of Collection (15)	2/5	6	5/5	15	4/5	12
Storage Requirements (10)	2/5	4	5/5	10	4/5	8
Total (100)	56		78		80	

Prior Detection:
The most important factor in which substance we would choose to test is how sure we could be that it would be able to accurately and efficiently produce a result for HPV. Since the accuracy will depend on our specific testing method and studies on detecting HPV in these substances vary in percent accuracy, the number we chose to base our decision on was how much the sample type had been previously used by other researchers.

Ease of Collection:
Each sample that can possibly be tested requires different methods of collection. The collection method should be the one that is easiest to obtain a sample from and require no prior knowledge of medical procedures.

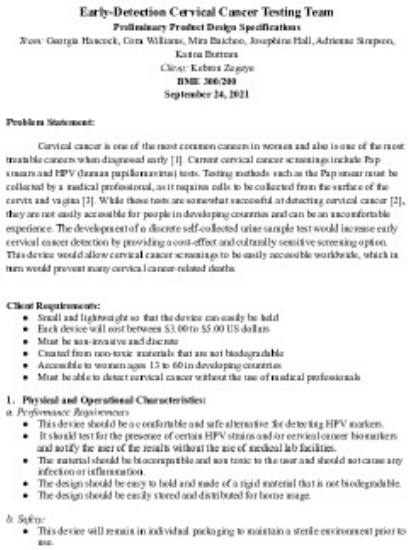
Comfort:
Patient comfort was an important consideration for this decision. Each sample medium collection process results in slightly different amounts of pain for the patient. The pain level of the patient should be minimal to non-existent while collecting the sample.

Ease of Obtaining Usable Sample: Different sample mediums can require slight variations in collection in order to obtain a usable sample. The sample mediums should not require prior planning or complex techniques for the collected sample to be usable.

Design_Martix.docx(10 KB) - download

10/19/2021 - PDS

MIRA BAICHOO - Oct 19, 2021, 5:58 PM CDT



Early-Detection Cervical Cancer Testing Team
Preliminary Product Design Specifications
Team: Georgia Hancock, Cora Williams, Mira Baichoo, Josephine Hall, Adrienne Simpson, Karina Buttram
Client: Kehinde Ziegler
BME 300/200
September 24, 2021

Problem Statement:

Cervical cancer is one of the most common cancers in women and also is one of the most treatable cancers when diagnosed early [1]. Current cervical cancer screenings include Pap smears and HPV (human papillomavirus) tests. Testing methods such as the Pap smear must be collected by a medical professional, as it requires cells to be collected from the surface of the cervix and vagina [3]. While these tests are somewhat successful at detecting cervical cancer [2], they are not easily accessible for people in developing countries and can be an uncomfortable experience. The development of a discrete self-collected urine sample test would increase early cervical cancer detection by providing a cost-effect and culturally sensitive screening option. This device would allow cervical cancer screenings to be easily accessible worldwide, which in turn would prevent many cervical cancer-related deaths.

Client Requirements:

- Small and lightweight so that the device can easily be held
- Each device will cost between $3.00 to $5.00 US dollars
- Must be non-invasive and discrete
- Created from non-toxic materials that are not biodegradable
- Accessible to women ages 13 to 60 in developing countries
- Must be able to detect cervical cancer without the use of medical professionals

1. Physical and Operational Characteristics:

a. Performance Requirements

- This device should be a comfortable and safe alternative for detecting HPV markers.
- It should test for the presence of certain HPV strains and/or cervical cancer biomarkers and notify the user of the results without the use of medical lab facilities.
- The material should be biocompatible and non toxic to the user and should not cause any infection or inflammation.
- The design should be easy to hold and made of a rigid material that is not biodegradable.
- The design should be easily stored and distributed for home usage.

b. Safety:

- This device will remain in individual packaging to maintain a sterile environment prior to use.

PDS_-_Cervical_Cancer_Testing_1_.pdf(103.7 KB) - download

MIRA BAICHOO - Oct 19, 2021, 5:57 PM CDT

Title: PDS

Date: September 24, 2021

Content by: Entire Group

Present: n/a

Goals: To list what our requirements and specifications are for this project given to us by our client.

Content:

The PDF is attached above.

Conclusions/action items: Continue to update the PDS if client requirements and specifications change.

10/19/2021 - Design Matrix 2

ADRIENNE SIMPSON - Oct 19, 2021, 6:01 PM CDT

Title: Design Matrix 2 (Collection Device)

Date: 10/19/21

Content by: BME design team

Present: BME design team

Goals:

To determine which collection method we would use for testing the sample

Content:

Designs / **Categories**	**#1 Strip Dip**		**#2 Drop Test**		**#3 Funnel Device**	
Ease of Use (30)	3/5	18	4/5	24	**5/5**	**30**
Cost (25)	**5/5**	**25**	3/5	15	2/5	10
Ease of Fabrication (20)	**5/5**	**20**	4/5	16	2/5	8
Sample Containment (15)	3/5	9	**5/5**	**15**	2/5	6
Efficiency (10)	4/5	10	**5/5**	**10**	3/5	6
Total (100)	**82**		**80**		60	

Conclusions/action items:

The Drop Test collection device is the method we decided on for testing the sample.

Designs Categories	#1 Strip Dip 		#2 Deep Test 		#3 Funnel Device 	
Ease of Use (30)	3/5	18	4/5	24	**5/5**	**30**
Cost (25)	**5/5**	**25**	3/5	15	2/5	10
Ease of Fabrication (20)	**5/5**	**20**	4/5	16	2/5	8
Sample Containment (15)	3/5	9	**5/5**	**15**	2/5	6
Efficiency (10)	4/5	10	**5/5**	**10**	3/5	6
Total (100)	**82**		10		60	

Ease of Use: - Josie

The criteria with the highest weight and importance is the ease of use. The user must be able to understand how the device is used and easily perform the desired tasks to collect a sample for testing.

Cost: - Mira

The criteria for cost is the second highest weight because the main point of this project is for women in rural areas to have access to a non-invasive method to detect cervical cancer. The product must cost the least amount of money to manufacture, which would allow it to be sold for the targeted price of $3-$5.

Ease of Fabrication: - Adrienne

We ranked ease of fabrication for each design based on which one would be the easiest to make and limited product malfunction. The device needs to be relatively easy so that the cost of manufacturing is lower thus making it easy to achieve our goal selling price for the product.

Design_Martix_Collection_Device_.pdf(1021.5 KB) - download

10/15/2021 - Preliminary Presentation

Josephine HALL (jrhall3@wisc.edu) - Oct 19, 2021, 5:57 PM CDT

Title: Preliminary Presentation

Date: 10/19/2021

Content by: Josephine Hall

Present: BME Design Team

Goals: Document the creation of the preliminary presentation

Content:

See PDF

Conclusions/action items: The preliminary presentation has been created and the team will now be working on the preliminary deliverables.

Josephine HALL (jrhall3@wisc.edu) - Oct 19, 2021, 5:57 PM CDT

BME_200_300_Preliminary_Design_Presentation.pdf(1.9 MB) - download

12/14/2021 - Poster Presentation

Josephine HALL (jrhall3@wisc.edu) - Dec 14, 2021, 5:30 PM CST

Title: Poster Presentation

Date: 12/14/2021

Content by: Josephine Hall

Present: Design Team without Mira

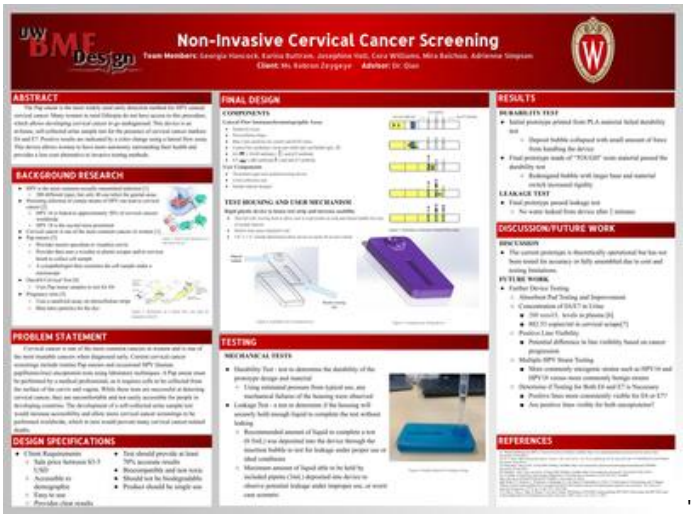
Goals: Document the Poster Presentation

Content:

See Image

Conclusions/action items: Poster Presentation has been completed and team will now move on to writing the final report

Josephine HALL (jrhall3@wisc.edu) - Dec 14, 2021, 5:30 PM CST



CoraWilliams-BME.pptx.jpg(688.7 KB) - download

12/14/2021 Updated PDS

KARINA BUTTRAM - Dec 14, 2021, 5:41 PM CST

Title: Updated Product Design Specifications

Date: 12/14/2021

Content by: Team

Present: Team

Goals: upload our updated PDS

Content:

Early-Detection Cervical Cancer Testing Team

Preliminary Product Design Specifications

Team: Georgia Hancock, Cora Williams, Mira Baichoo, Josephine Hall, Adrienne Simpson, Karina Buttram

Client: Kebron Zegeye

BME 200/300

December 15, 2021

Problem Statement:

Cervical cancer is one of the most common cancers in women and also is one of the most treatable cancers when diagnosed early [1]. Current cervical cancer screenings include routine Pap smears and occasional HPV (human papillomavirus) oncoprotein tests using laboratory techniques. A Pap smear must be performed by a medical professional, as it requires cells to be collected from the surface of the cervix and vagina. While these tests are successful at detecting cervical cancer, they are uncomfortable and not easily accessible for people in developing countries. The development of a self-collected urine sample test would increase accessibility and allow more cervical cancer screenings to be performed worldwide, which in turn would prevent many cervical cancer-related deaths.

Client Requirements:

- Small and lightweight so that the device can easily be held
- Each device will cost between $3.00 to $5.00 US dollars
- Must be non-invasive and discrete
- Created from non-toxic materials that are not biodegradable
- Accessible to women ages 13 to 60 in developing countries
- Must be able to detect cervical cancer without the use of medical professionals

1. Physical and Operational Characteristics:

a. Performance Requirements

- This device should be a comfortable and safe alternative for detecting cervical cancer biomarkers.
- It should test for the presence of cervical cancer biomarkers and notify the user of the results through a color change without the use of medical lab facilities.
- The material should be biocompatible and non toxic to the user and should not cause any infection or inflammation.

- The design should be easy to hold and made of a rigid material that is not biodegradable.
- The design should be easily stored and distributed for home usage.

b. Safety:

- This device will remain in individual packaging to maintain a sterile environment prior to use.
- It should be biocompatible with no toxic materials and not cause any infections or inflammation.

c. Accuracy and Reliability:

- This device should be able to detect cervical cancer biomarkers from a sample collected at home. It should produce at least 70% accurate results.

d. Life in Service:

- The device should be disposed of after each use.

e. Shelf Life:

- This device should be stored in sealed, sterile packaging prior to use. The device will operate in temperatures ranging from 50$_{o}$F-110$_{o}$F. It will have a shelf life of approximately 1-3 years, while remaining in a sealed package [2].

f. Operating Environment:

- The device is designed to be used by women in developing countries in a non-medical environment.
- The device will provide clear instructions to conduct the test in any setting with no other equipment necessary.

g. Ergonomics:

- The device will be small and lightweight so that it may easily be held.

h. Size:

- The device will be 3” long and 1.5” wide.

i. Weight:

- The device weighs 0.5 oz.

k. Materials:

- Materials in contact with the user during sample collection are biocompatible
- No materials used are biodegradable

l. Aesthetics, Appearance, and Finish:

- This device is compact so that it can be easily be held in the users hand
- Results should be easy to read and use no words so that users who speak any language can read the results universally
- Test should be discrete in appearance to avoid taboos around women's health

2. Product Characteristics

a. Quantity:

- One sample collection cup
- One 3mL pipette
- One biomarker testing device

b. Target Product Cost:

- The device should cost between $3-$5 per test to manufacture.

3. Miscellaneous

a. Standards and Specifications:

- Does not require a doctor or other healthcare professionals for collection or interpretation of results
- Discrete packaging
- Clear indicator of positive or negative results

b. Patient-Related Concerns:

- This product is designed for women in rural areas who do not have access to doctors or healthcare. The test must be easy to use and available for women in cultures where women's health topics are not discussed. It is important that the product will provide a clear answer if the patient has cervical cancer markers or not, this way the patient can make an informed decision toward next steps and receive medical care.

c. Competition:

- Currently there is no non-invasive method for testing for cervical cancer. The main way for testing is a Pap Smear, which is a very invasive method, requires a doctor, and can be very expensive. A Pap Smear, is where a provider inserts a speculum to visualize the cervix and uses a wooden or plastic scraper and/or cervical brush to collect cell samples[3]. This method helps screen for abnormal cells that have the ability to turn into cervical cancer.
- The OncoE6 Cervical Test is a rapid and easy-to-use test based on the detection of E6 oncoproteins from high risk HPV types 16 and 18 using highly specific monoclonal antibodies (mAbs) in a lateral-flow (LF) assay format. It is available in the US, as a service through our CLIA-certified laboratory. This qualitative test is used to analyze cells extracted from cervical cytology swab specimens [4].
- Another method used in more rural parts of Africa and India is Visual Inspection with Acetic Acid(VIA). The procedure is similar to a Pap Smear except a 5% solution of acetic acid is swabbed onto the cervix and left there for 60 seconds. After the time has passed, a precancerous lesion will turn white with clear and dense margins, this is considered a positive result [5]. After a positive result the patient would be referred for further treatment.

References

[1] “Cervical cancer,” *World Health Organization*. [Online]. Available: https://www.who.int/health-topics/cervical-cancer#tab=tab_1. [Accessed: 23-Sep-2021].

[2] the H. E. Team, “Do pregnancy tests expire? what to know before using one,” Healthline, 16-Dec-2019. [Online]. Available: https://www.healthline.com/health/pregnancy/do-pregnancy-tests-expire. [Accessed: 12-Dec-2021].

[3] “HPV and PAP testing,” *National Cancer Institute*, 20-Dec-2019. [Online]. Available: https://www.cancer.gov/types/cervical/pap-hpv-testing-fact-sheet#what-is-cervical-cancer-screening. [Accessed: 23-Sep-2021].

[4] “OncoE6 Cervical Test,” Arbor Vita Corporation, 25-May-2020. [Online]. Available: https://www.arborvita.com/oncoe6/. [Accessed: 03-Nov-2021].

[5] U. R. Poli, P. D. Bidinger, and S. Gowrishankar, “Visual inspection with acetic acid (VIA) screening program: 7 years experience in early detection of cervical cancer and pre-cancers in rural South India,” *Indian Journal of Community Medicine*, vol. 40, no. 3, p. 203, 2015.

Conclusions/action items: This document is our updated product design specifications that have slightly changed since our preliminary work.

12/14/2021 Final Report

Josephine HALL (jrhall3@wisc.edu) - Dec 14, 2021, 9:03 PM CST

Title: FInal Report

Date: 12/14/2021

Content by: Team

Present: Team

Goals: upload our final report

Content:

Conclusions/action items: This is our final report document.

Josephine HALL (jrhall3@wisc.edu) - Dec 14, 2021, 9:04 PM CST

Non-Invasive_Cervical_Cancer_Screening_-_Final_Report.pdf(4.4 MB) - download

9/29/21-HPV Detection in Different Sample Types

Georgia Hancock - Oct 04, 2021, 5:12 PM CDT

Title: HPV Detection in Different Sample Types

Date: 9/29/21

Content by: Georgia Hancock

Present: -

Goals: Gather data on what types of samples can produce an accurate HPV test result

Content:

-HPV can be accurately detected using a urine test as an alternative to pap smear testing

-Currently no blood tests available for HPV

-An accurate HPV sample can be detected from saliva, but is not necessarily indicative of a cervical cancer risk

Conclusions/action items:

Use information for sample type matrix

Sources:

"New study finds HPV can be detected through urine testing," *Carrington College*, 24-Feb-2021. [Online]. Available: https://carrington.edu/blog/new-study-finds-hpv-can-detected-urine-testing/. [Accessed: 04-Oct-2021].

"Diagnosis," *HPV Diagnosis & Detection | HPV DNA Tests Sometimes Used*. [Online]. Available: https://www.hpv.org.nz/hpv-diagnosis#:~:text=Unfortunately, there is no swab,an abnormal cervical smear result. [Accessed: 04-Oct-2021].

"Blood and Saliva Tests Help Predict Return of HPV-Linked Oral Cancers - 07/31/2014," *Johns Hopkins Medicine, based in Baltimore, Maryland*. [Online]. Available: https://www.hopkinsmedicine.org/news/media/releases/blood_and_saliva_tests_help_predict_return_of_hpv_linked_oral_cancers. [Accessed: 04-Oct-2021].

12/1/21 - Concentration of E6 and E7 Oncoproteins in Urine

Georgia Hancock - Dec 03, 2021, 1:00 PM CST

Title: Concentration of E6 and E7 Oncoproteins in Urine

Date: 12/1/21

Content by: Georgia Hancock

Present: Georgia Hancock

Goals: Explore current research on concentrations of these proteins in urine

Content:

E6 and E7 were detected in concentrations of 200 nmol/L in plasma

Conclusions/action items:

We may not be able to determine the concentrations present in urine, we would need to conduct clinical trial

References:

H. Reder, V. F. Taferner, C. Wittekindt, A. Bräuninger, E.-J. M. Speel, S. Gattenlöhner, G. Wolf, J. P. Klussmann, N. Wuerdemann, and S. Wagner, “Plasma cell-free human papillomavirus oncogene E6 and E7 DNA predicts outcome in oropharyngeal squamous cell carcinoma,” *The Journal of Molecular Diagnostics*, 18-Aug-2020. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S1525157820304293. [Accessed: 02-Dec-2021].

9/14/21-Current Cervical Cancer Testing Methods

Georgia Hancock - Sep 16, 2021, 11:23 AM C

Title: Current Cervical Cancer Testing Methods

Date: 9/14/21

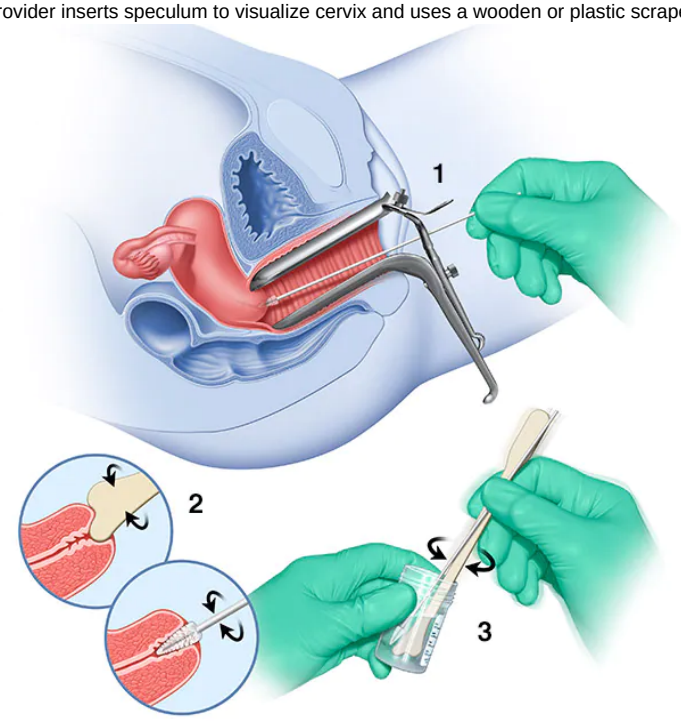
Content by: Georgia Hancock

Present: -

Goals: Understand current screening methods, including effectiveness and flaws

Content:

- Tested for during Pap Smear/ HPV test
  - Recommended by doctors for women 21-65
  - Every 3 years
  - Provider inserts speculum to visualize cervix and uses a wooden or plastic scraper and/or cervical brush to collect cell sample



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- Cervical intraepithelial neoplasia (CIN) is a condition of abnormal cells lining the cervix detectable by pap smear that are not cancerous but can progress into cervical cancer.
  - 3 stages
- Key is finding abnormal cells before cancer forms
- Pap smear risks (false negative):
  - Inadequate collection of cells
  - Small number of abnormal cells
  - Blood or inflammatory cells blocking abnormal cells
- Other issues with pap smears:
  - Can cause cramping and discomfort
  - Can cause bleeding

Sources:

- “HPV and Pap Testing,” *National Cancer Institute*. [Online]. Available: https://www.cancer.gov/types/cervical/pap-hpv-testing-fact-sheet#what-is-cervical-cancer-screening. [Accessed: 16-Sep-2021].
- American Cancer Society.Cancer Facts & Figures 2021. Atlanta: American Cancer Society, “Cervical Cancer,” *CancerQuest*. [Online]. Available: https://www.cancerquest.org/patients/cancer-type/cervical-cancer?gclid=Cj0KCQjwkIGKBhCxARIsAINMioLNxpbNs0IOW9N2RnYzO51lvg5tBtKcbOCJQedCaWfP0sKmTrIunWYaAstHEALw_wcB#detection-diagnosis. [Accessed: 16-Sep-2021].
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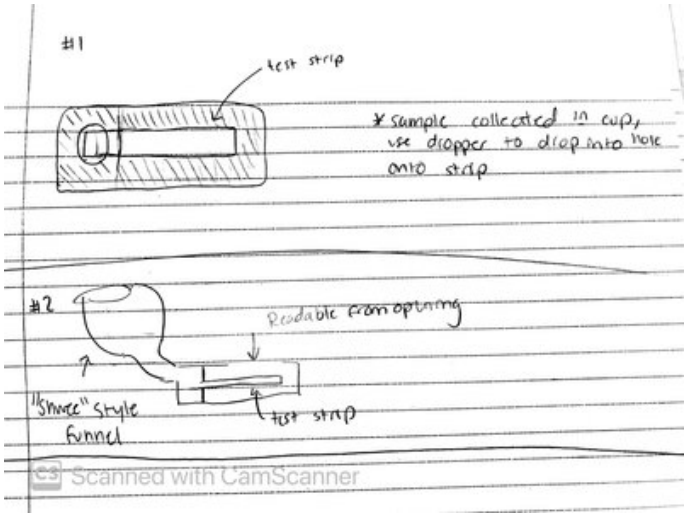
Conclusions/action items:

10/4/21 - Design Ideas

Georgia Hancock - Oct 04, 2021, 5:30 PM CDT



Design_Ideas.jpg(428.6 KB) - download

Georgia Hancock - Dec 14, 2021, 11:16 AM CST

Title: Design Ideas

Date: 10/4/21

Content by: Georgia Hancock

Present: Georgia Hancock, Adrienne Simpson, Mira Baichoo, Josephine Hall, Cora Williams

Goals:

Sketch design ideas and discuss with the team before building our design matrix

Content:

-Design one consists of a small rectangular testing apparatus with a hole to deposit sample onto absorbent pad as well as a viewing hole where the results of the sample will be visible to the user

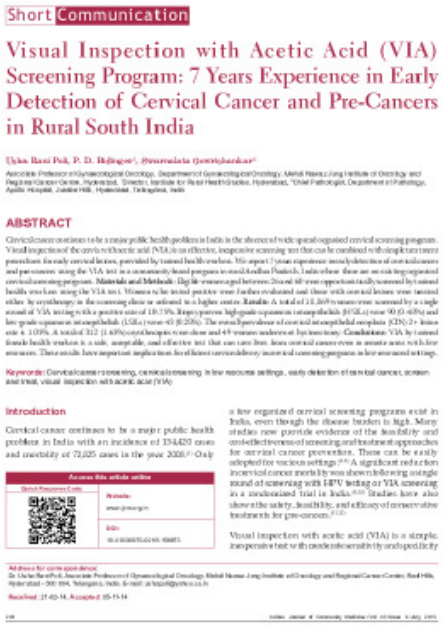
-Design 2 consists of a funnel device where the sample will be collected by the user into the funnel which will flow directly to the test strip in the housing below

Conclusions/action items:

complete design matrix

Research 09/17 Methods of Detection on Market Currently

MIRA BAICHOO - Oct 04, 2021, 5:27 PM CDT



Short Communication

Visual Inspection with Acetic Acid (VIA) Screening Program: 7 Years Experience in Early Detection of Cervical Cancer and Pre-Cancers in Rural South India

Usha Rani Peli, P. D. Rajnigee[1], Swarnalata Gorrigharkar[2]

Associate Professor in Gynaecological Oncology, Department of Gynaecological Oncology, Mahila Nawaz Jung Institute of Oncology and Regional Cancer Centre, Hyderabad, [1]Director, Institute for Rural Health Studies, Hyderabad, [2]Chief Pathologist, Department of Pathology, Apollo Hospital, Jubilee Hills, Hyderabad, Telangana, India

ABSTRACT

Cervical cancer continues to be a major public health problem in India as the absence of widespread organized cervical screening programs. Visual inspection of the cervix with acetic acid (VIA) is an effective, inexpensive screening test that can be combined with single treatment procedure for early cervical lesions, provided by trained health workers. We report 7 years experience in early detection of cervical cancers and pre-cancers using the VIA test in a community-based program in rural Andhra Pradesh, India where there are no existing organized cervical screening programs. **Materials and Methods:** Eligible women aged between 20 and 60 years opportunistically screened by trained health workers using the VIA test. Women who tested positive were further evaluated and those with cervical lesions were treated either by cryotherapy in the screening clinic or referred to a higher centre. **Results:** A total of 11,869 women were screened by a single round of VIA testing with a positive rate of 10.73%. Biopsy proven high-grade squamous intraepithelial lesion (HSIL) was 90 (0.68%) and low-grade squamous intraepithelial (LSIL) were 43 (0.32%). The overall prevalence of cervical intraepithelial neoplasia (CIN) 2+ lesions was 1.03%. A total of 312 (1.63%) cryotherapies were done and 49 women underwent hysterectomy. **Conclusions:** VIA by trained female health workers is a safe, acceptable, and effective test that can save lives from cervical cancer even in remote areas with low resources. These results have important implications for future cervical cancer screening programs in low resourced settings.

Keywords: Cervical cancer screening, cervical screening, in low resource settings, early detection of cervical cancer, screen and treat, visual inspection with acetic acid (VIA)

Introduction

Cervical cancer continues to be a major public health problem in India with an incidence of 134,420 cases and mortality of 72,825 cases in the year 2008.[1] Only a few organized cervical screening programs exist in India, even though the disease burden is high. Many studies now provide evidence of the feasibility and cost-effectiveness of screening and treatment approaches for cervical cancer prevention. These can be easily adopted for resource settings.[2-5] A significant reduction in cervical cancer mortality was shown following a single round of screening with HPV testing or VIA screening in a randomized trial in India.[6,7] Studies have also shown the safety, feasibility, and efficacy of conservative treatments for pre-cancers.[8-12]

Visual inspection with acetic acid (VIA) is a simple, inexpensive test with moderate sensitivity and specificity

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Received: 21-03-14, Accepted: 05-11-14

VIA_PDF_.pdf(362.7 KB) - download

MIRA BAICHOO - Oct 04, 2021, 5:26 PM CDT

Title: Research on Methods of Detection on Market Currently

Date: 9/17/2021

Content by: Mira Baichoo

Present: n/a

Goals: Know more about what is on the market and what low-income countries are doing instead of normal pap smears

Content:

- A speculum is used to get a visualization of the cervix
- A 5% acetic acid solution is used
- A cotton swab drenched in the acetic acid goes into the vagina and is it swabbed onto the cervix
    - The swab is left on the cervix for 60 seconds
    - A precancerous lesion will turn white or acetowhite with clear and dense margins on the SCJ (squamo columnar junction) this is considered a positive result
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4478664/
- https://vimeo.com/81485387 (informative video)

Citation: U. R. Poli, P. D. Bidinger, and S. Gowrishankar, “Visual inspection with acetic acid (VIA) screening program: 7 years experience in early detection of cervical cancer and pre-cancers in rural South India,” *Indian Journal of Community Medicine*, vol. 40, no. 3, p. 203, 2015.

Conclusions/action items:

N/A

Urinary Biomarkers for the diagnosis of Cervical Cancer

KARINA BUTTRAM - Oct 26, 2021, 3:13 PM CDT

Title: Urinary Biomarkers for the diagnosis of cervical cancer by quantitative label-free mass spectrometry analysis

Date: 9/18/2021

Content by: Karina Buttram

Link: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6507435/pdf/ol-17-06-5453.pdf

Citation: D. Chokchaichamnankit, K. Watcharatanyatip, P. Subhasitanont, et al. "Urinary biomarkers for the diagnosis of cervical cancer by quantitative label-free mass spectrometry analysis," *Oncology Letters, 17,5453-5468, 2019. Available:* DOI: 10.3892/ol.2019.10227. [Sept. 18, 2021].

Present: Karina Buttram

Goals: introduction to markers in urine commonly seen with cervical cancer

Content:

-non-invasive tests has many benefits for developing countries

-notable urinary proteins: LRGI, MMRN1, S100A8, SERPINB3, CD44

-"cervical cancer is the fourth most frequent cause of mortality in women worldwide"

-precancerous state of cervical cancer is known as cervical intraepithelial neoplasia (CIN), high risk HPV typically associated with cervical cancer

-cervical cancer is treated with typical cancer treatments such as chemotherapy, surgery, radiation therapy

-typical tests for cervical cancer is a pap smear (most common), liquid-based cytology and HPV DNA testing

-research on chromatography for a non invasive test

-in this study, measure protein concentrations from urine samples using spectrophotometry

-results: found that LRGI, MMRN1, S100A8, SERPINB3, and CD44 were the notable proteins from the urine samples, COULD BE POTENTIAL BIOMARKERS FOR CERVICAL CANCER

-21 proteins they found from urine samples were already associated with cervical cancer, but 112 proteins they found in the samples have never been associated with cervical cancer database yet

Conclusions/action items: There has been some research into urinary biomarkers for cervical cancer, but there is not a single determine biomarker yet. This study used mass spectrometry to look at possible proteins that could be biomarkers in urine; however, 5 proteins stood out among others. LRGI, MMRN1, S100A8,SERPINB3, and CD44 are the most notable proteins from this study. Most of the proteins found in this study have yet to be associated with cervical cancer, so one single biomarker in urine for cervical cancer is still being determined.

E6 Oncoprotein to Drosophila discs

KARINA BUTTRAM - Nov 03, 2021, 5:26 PM CDT

Title: Binding of high-risk human papillomavirus E6 oncoproteins to the human homologue of the *Drosophila* discs large tumor suppressor protein

Date: 10/26/2021

Content by: Karina Buttram

Citation: T. Kiyono, A. Hiraiwa, M. Fujita, et al. **"**Binding of high-risk human papillomavirus E6 oncoproteins to the human homologue of the *Drosophila* discs large tumor suppressor protein," *Proceeding of the National Academy of Sciences of the United States of America*, 94(21),11612-11616, 1997. Available: https://doi.org/10.1073/pnas.94.21.11612. [October 26, 2021].

Present: Karina Buttram

Goals: determine if the E6 oncoprotein is a good biomarker for our project

Content:

- E6 can bind to the hDLG protein during the cancer development

Conclusions/action items:

Recap only the most significant findings and/or action items resulting from the entry.

Functions of E6

KARINA BUTTRAM - Nov 03, 2021, 5:26 PM CDT

Title: Novel Functions of the HPV E6 oncoprotein

Date: 10/26/21

Content by: Karina Buttram

Citation: N. A. Wallace, D. A. Galloway. "Novel Functions of the HPV E6 oncoprotein," *The Annual Review of Virology*, 2,403-423, 2015. Available DOI: 10.1146/annurev-virology-100114-055021. [October, 26, 2021].**Present:** Karina Buttram

Goals: understand more about E6

Content:

- infection begins in basal layer of epithelia -> further infection and expression of HPV E6 and E7

- E6 promotes p53 degradation and causes the activation of telomerase (elongates chromosomes, causes cells to age)

- E6 extends life span of cells thru telomerase and allows for oncogenesis that degrades p53 (a tumor suppressor protein)

- can be present 20-30 years before it is detected

- KEY FUNCTIONS: degradation of p53, telomerase activation, transformation of host cells with high levels of E6 present

- causes an increase in miRNA-218, 23b, 24, 205, and 203 levels and LAMB3

- disrupts G protein signaling

Conclusions/action items: E6 focuses on the degradation of p53 and activates telomerase.

Fluorescent spectra of blood and urine for cervical cancer detection

KARINA BUTTRAM - Oct 26, 2021, 3:15 PM CDT

Title: Fluorescent spectra of blood and urine for cervical cancer detection

Date: 10/1/21

Content by: Karina Buttram

Link: https://www.spiedigitallibrary.org/journals/journal-of-biomedical-optics/volume-17/issue-9/098001/Fluorescence-spectra-of-blood-and-urine-for-cervical-cancer-detection/10.1117/1.JBO.17.9.098001.full?SSO=1

Citation: V. Masilamani, M. S AlSalhi, T. Vijmasi, K Govindarajan, R R Rai, M. Atif, S Prasad, A Aldwayyan. "Fluorescence spectra of blood and urine for cervical cancer detection," *SPIE Digital Library,* 17(9), 2012. Available: https://www.spiedigitallibrary.org/journals/journal-of-biomedical-optics/volume-17/issue-9/098001/Fluorescence-spectra-of-blood-and-urine-for-cervical-cancer-detection/10.1117/1.JBO.17.9.098001.full?SSO=1. [Oct. 1, 2021].

Present: Karina Buttram

Goals: understand how using fluorescent spectroscopy could be a form of collection for us

Content:

-FES: fluoresence emission spectroscopy, SSS: stokes shift spectra

-using FES and SSS to show signs of cervical cancer

-both FES and SSS for urine showed differences among cervical cancer patients and non cervical cancer patients (higher intensity indicated cervical cancer)

-urine samples that were normal were a pale green color, urine samples that were cancerous were yellowish or yellowish-red

-FES shows differences in fluorophores such as porphyrin and flavin in cancer patients

-very simple and cost-effective way to test

-acetone brings out the fluorophores from the inside of cells

Conclusions/action items: We could potentially have people ingest some kind of fluorescent molecule to change the color of their urine to indicate if they have cervical cancer or not. This study used FES to determine this and it was successful, but I'm not exactly sure what fluorescent was used in this study. This would be a non-invasive and cost effective way to test people for cervical cancer.

Cervical cancer detection by DNA methylation analysis in urine

KARINA BUTTRAM - Oct 26, 2021, 3:15 PM CDT

Title: cervical cancer detection by DNA methylation analysis in urine

Date: 10/1/21

Content by: Karina Buttram

Link: https://www.nature.com/articles/s41598-019-39275-2

Citation: B.C. Snoek, A. P. V. Splunter, M. C. G Bleeker. "Cervical cancer detection by DNA methylation analysis in urine," *Scientific reports,* 9(3088), 2019. Available: https://doi.org/10.1038/s41598-019-39275-2. [Oct. 1, 2021].

Present: Karina Buttram

Goals: understand how DNA methylation shows as a cervical cancer marker in urine

Content:

-study testing for high-risk HPV (HrHPV)

- DNA was isolated from a urine sample and tested using an EZ DNA Myelination kit (a kit for a rxn btwn cytosine and sodium bisulfite which converts cytosine to uracil)

-there are 6 DNA myelination markers in urine for cervical cancer: (*FAM19A4*, *GHSR*, *PHACTR3*, *PRDM14*, *SST*, and *ZIC1*)

- majority of tests in this trial detected HPV 16 and 18

-urine sediment (cells and crystals) vs. native urine samples gave roughly the same outcomes, but slightly better outcomes from urine sediment -> continued results with urine sediment

-urine sediment tests showed "nearly-perfect" results compared to the pap smear tests

Conclusions/action items: DNA methylation could be a technique that we use in our test. I will need to further research the reaction between DNA and sodium bisulfate to see if that is a possible reagent we could use to indicate a color change on our design. Urine sediment collection shows nearly the same outcomes as pap smear tests, so we know that urine testing is a viable collection method for this project.

IgG and IgA in Blood

KARINA BUTTRAM - Nov 03, 2021, 5:29 PM CDT

Title: Risk Factors for Subsequent Cervicovaginal Human Papillomavirus (HPV) Infection and the Protective Role of Antibodies to HPV-16 Virus-Like Particles

Date: 10/26/21

Content by: Karina Buttram

Citation: G. Y. F. Ho, Y. Studentsov, C. B. Hall, et al. "Risk Factors for Subsequent Cervicovaginal Human Papillomavirus (HPV) Infection and the Protective Role of Antibodies to HPV-16 Virus-Like Particles," *The Journal of Infectious Diseases*, 186(6),737-742, 2002. Available: https://doi.org/10.1086/342972. [October 26, 2021].

Present: Karina Buttram

Goals: further understand IgG and IgA antibodies

Content:

- study: 17-23 year olds of different races, took blood tests at intial visits and follow-up visits that were all six months apart

- IgG was present in 65%, IgA was present in 24% (IgA showed lower circulation levels than IgA)

- presence of IgG and IgA showed positive results for cervical cancer

- 69.4% of samples positive for IgA also positive for IgG, only 10.4% of samples negative for IgA were positive for IgG

- IgG positive level was a titer>400-800

- samples with both IgG and IgA had a 53% reduced risk of infection compared to samples with just IgG

samples with both IgG and IgA showed most accurate results

Conclusions/action items: Looking for both IgG and IgA antibodies would give us the most accurate results, but this test was done in blood. Theoretically, these antibodies are also present in urine, so this should still work for our project.

IgA in urine

KARINA BUTTRAM - Nov 03, 2021, 5:31 PM CDT

Title: IgA- containing immune complexes in the urine of IgA nephropathy patients

Date: 10/26/21

Content by: Karina Buttram

Citation: K. MAtousovic, J. Novak, T. Yanagihara, et al. "IgA- containing immune complexes in the urine of IgA nephropathy patients," *Nephrology Dialysis Transplantation*, 21(9),2478-2484, 2006. Available: https://doi.org/10.1093/ndt/gfl240. [October 26, 2021].

Present: Karina Buttram

Goals: determine in IgA is present in urine and will be a good biomarker

Content:

- measured IgA and IgG concentrations in urine of renal disease patients using ELISA (enzyme-linked immunosorbent assay)

- levels usually increase with disease progression bc immune complexes produce more antibodies

- renal diseased patients showed drastically higher levels of IgA than healthy patients (concentration of 24.2-53.4 vs. 2.5-4.3 in healthy patients) and IgG (concentration of 12.3-22.1 vs. 10.5-21.0 in healthy patients)

- IgG and IgA immune complexes had much higher levels in diseased samples

- both antibodies found in urine samples

-

Conclusions/action items: IgA was found in higher concentrations than IgG in the urine samples. Both antibodies were still present in the samples.

IgG and IgA in HPV positive samples

KARINA BUTTRAM - Nov 03, 2021, 5:34 PM CDT

Title: Occurrence of IgA and IgG Antibodies to Select Peptides Representing Human Papillomavirus Type 16 among Cervical Cancer Cases and Controls

Date: 10/26/21

Content by: Karina Buttram

Citation: V. M. Mann, S. L. Lao, M. Brenes, et al. "Occurrence of IgA and IgG Antibodies to Select Peptides Representing Human Papillomavirus Type 16 among Cervical Cancer Cases and Controls," *Cancer Research*, 50,7815-7819, 1990. Available: https://cancerres.aacrjournals.org/content/50/24/7815.full-text.pdf. [October 26, 2021].

Present: Karina Buttram

Goals: determine which antibody is present in the highest concentration in HPV patients

Content:

- urine serum samples from women who are HPV positive and have had no cervical cancer treatment, using ELISA

- this study in relation to E7 peptide and 245 peptide

- most accurate results shown with IgG in E7

-found IgG and IgA with E7 and only IgG with peptide 245

- no coorelation btwn results and stage of disease

- 31% of HPV-16 positive samples had IgA, 60% of HPV-16 positive samples showed both IgA and IgG

Conclusions/action items: Both antibodies are present in urine for women with HPV-16. Both antibodies present showed more accurate results than just testing for one of the antibodies.

HPV 16, 18 and 6

KARINA BUTTRAM - Nov 03, 2021, 5:36 PM CDT

Title: Comparison of Human Papillomavirus Types 16, 18, and 6 Capsid Antibody Responses Following Incident Infection

Date: 10/27/21

Content by: Karina Buttram

Citation: J. J. Carter, L. A. Koutsky, J. P. Hughes, et al. "Comparison of Human Papillomavirus Types 16, 18, and 6 Capsid Antibody Responses Following Incident Infection," *The Journal of Infectious Diseases*, 181(6),1911-1919, 200. Available: https://doi.org/10.1086/315498. [October 27, 2021].

Present: Karina Buttram

Goals: further understand HPV 16 and 18 antibodies

Content:

-study done during seroconversion period where antibodies are then detectable

- highest percentage was 56% positive results for HPV-16 and only 36.4% positive results for HPV-18

- HPV-6 was detected far less frequently than HPV 16 or 18

- HPV-16 found more often than HPV-6 after individual had a new sex partner

- rare to have multiple different strained of HPV

-these tests were not sensitive enough to detect low levels of antibodies

-roughly 60% of patients in this study hit seroconversion period within 18 months of initial detection of HPV antibodies

Conclusions/action items: HPV 16 and 18 are the most prevalent HPV antibodies; however, it is difficult to detect these antibodies at low concentrations using lab techniques, so this would be difficult to test at home.

E7 vs. low-risk HPV strains

KARINA BUTTRAM - Nov 03, 2021, 5:38 PM CDT

Title: Biochemical and Biological Differences between E7 Oncoproteins of the High and Low Risk Human Papillomavirus Types are determined by amino-terminal sequences

Date: 11/2/2021

Content by: Karina Buttram

Present: Karina Buttram

Citation: K. Munger, C. L. Yee, W. C. Phelps, et al. "Biochemical and Biological Differences between E7 Oncoproteins of the High and Low Risk Human Papillomavirus Types are determined by amino-terminal sequences," *Journal of Virology*, 65(7),3943-3948, 1991. Available:https://journals.asm.org/doi/epdf/10.1128/jvi.65.7.3943-3948.1991. [November 2, 2021].

Goals: determine why it is more important to look at E6 and E7 than other oncoproteins

Content:

- HPV-16 vs. HPV-6 E7

- hpv-18 and hpv-16 are the only two strains considered high-risk, develop into carcinomas 85% of the time

- E6 and E7 are expressed in all HPV genomes and together they transform epithelial cells into cancerous cells

- neither oncoproteins have any known enzymatic functions

-E6 binds with p53 (a tumor suppressor protein)

-E7 can bind to a nuclear 21-kDa phosphoprotein

- E7 is associated with pRB (protein encoded by retinoblastoma susceptibility gene, a tumor suppressor protein)

- E7 better bind with pRB encoded by high-risk strains of HPV than low-risk strains

Link: https://journals.asm.org/doi/epdf/10.1128/jvi.65.7.3943-3948.1991

Conclusions/action items:

We should test for both E6 and E7 given that both attach to important tumor suppressor proteins in order for HPV to further develop. This is more prevalent in high-risk HPV strains, once again reassuring us that we should focus more on HPV 16 and 18 than other low-risk strains.

p53 peptide sequencing

KARINA BUTTRAM - Nov 03, 2021, 5:41 PM CDT

Title: Investigating peptide sequence variations for "double-click" stapled p53 peptides

Date: 11/2/2021

Content by: Karina Buttram

Present: Karina Buttram

Citation: Y. H. Lau, P. Andrade, N. Skold, et al. "Investigating peptide sequence variations for "double-click" stapled p53 peptides," *Organic and Biomolecular Chemistry*, 12,4074-4077, 2014. Available: https://pubs.rsc.org/en/content/articlehtml/2014/ob/c4ob00742e?casa_token=T_Sccr9EUB0AAAAA:NoI_Xv5NFE4xY_eoboyiiJJCCNCtwwM5j_z9Uf0tR9oZ-qiCGwTQevD8iYTp4BpQMeuwNBvAuOjFQ7w. [November 2, 2021].

Goals: understand how we could coat a test strip in p53 for E6 to bind to

Content:

- made the peptide sequence longer by stapling hydrocarbons to form an alpha-helix shape

- stapling improves the binding affinity of the peptides

- used other forms of peptide macrocyclisation for different stapling techniques

- "We have developed a double-click method of stapling peptides in solution,[7] where linear diazidopeptides are reacted with dialkynyl linkers to create bis-triazole stapled peptides under Cu(I) catalysis,[8] without the need for protecting groups (Fig. 1)."

- study done in vitro to inhibit certain protein-proteininteractions

Link: https://pubs.rsc.org/en/content/articlehtml/2014/ob/c4ob00742e?casa_token=T_Sccr9EUB0AAAAA:NoI_Xv5NFE4xY_eoboyiiJJCCNCtwwM5j_z9Uf0tR9oZ-qiCGwTQevD8iYTp4BpQMeuwNBvAuOjFQ7w

Conclusions/action items: Peptide stapling is something we could potentially look more into for coating the test strip in a tumor suppressor protein. The stapling of the p53 peptide sequence improves its ability to bind to other biomarkers, so this could be a technique that could potentially speed up the bind of p53 to E6.

E6 and E7 role in carcinogenesis

KARINA BUTTRAM - Nov 03, 2021, 5:59 PM CDT

Title: The role of HPV E6 and E7 Oncoproteins in HPV-associated Cervical Carcinogenesis

Date: 11/3/2021

Content by: Karina Buttram

Present: Karina Buttram

Citation: E. K. Yim, J. S. Park. "The role of HPV E6 and E7 Oncoproteins in HPV-associated Cervical Carcinogenesis," *Cancer Research and Treatment,* 37(6),319-324, 2005. Available:10.4143/crt.2005.37.6.319. [November 3, 2021].

Goals: further understand the roles of E6 and E7 and how they develop

Content:

- HPV-16 and 18 have double stranded DNA genomes and encode eight genes, some of which code for E6 and E7

- e6 and e7 must be present for malignant conversion, and associate with p53 and pRB

- E6 promotes cell proliferation by causing the degradation of p53 by forming E6-AP, this disrupts the cell cycle and leads to increase tumor cell growth

- E6 also has roles independent of p53

- E7 bind to pRb causing these cells to cluster in "pocket domains", pRb then binds to E2F transcription factors a suppresses the replication of enzyme genes -> rapid cell division

Host proteins associated with HPV E6 Oncoprotein

- E6 targets E3 ubiquitin ligase E6AP to p53 which will mark p53 for degradation

- regulated the replication of proteins involved in apoptosis and immune evasion

- "TRAF-interacting protein (I-TRAF) has been shown to be up-regulated by E6"

Host proteins associated with HPV E7 Oncoprotein

- E7 binding to pRb enhanced phosphorylation and degradation

- target types of cyclin and other transcription factors

Conclusions/action items: E6 and E7 will bind to E6AP and E2F to alter DNA sequences and promote rapid tumor cell growth. I need to further research these new biomarkers and determine if their peptide sequences could be used on our test strip.

p53 in Urine Sediment

KARINA BUTTRAM - Nov 04, 2021, 10:35 PM CDT

Title: Clinical implications of p53 mutation analysis in bladder cancer tissue and urine sediment by functional assay in yeast

Date: 11/4/2021

Content by: Karina Buttram

Present: Karina Buttram

Citation: B. Schlichtholz, M. Presler, M. Matuszewski. "Clinical implications of p53 mutation analysis in bladder cancer tissue and urine sediment by functional assay in yeast," *Carcinogenesis,* 12,2319-2323. Available: 10.1093/carcin/bgh256. [November 4, 2021].

Goals: determine that p53 can be found in urine

Content:

- for patients with carcinomas in bladder

- 80% of urine sediment samples had p53 mutations in it

- p53 mutations in urine sediment indicated a more advanced stage of cancer

Conclusions/action items: p53 can be found in urine sediment. This may require us to use a filter in our device to filter the urine sediment from the urine, but more research will need to be done regarding how to filter the urine.

p53 Interaction with DNA

KARINA BUTTRAM - Nov 04, 2021, 11:02 PM CDT

Title: Modes of p53 interaction with DNA in the chromatin context

Date: 11/4/2021

Content by: Karina Buttram

Present: Karina Buttram

Citation: V. Vukojevic, T. Yakovleva, G. Bakalkin. "Modes of p53 interaction with DNA in the chromatin context," *NCBI.* Available: https://www.ncbi.nlm.nih.gov/books/NBK6238/. [November 4, 2021].

Goals: learn more about p53 role in DNA sequencing

Content:

- "The p53 binding site consists of two half-sites 5´-PuPuPuC(A/T)(T/A)GPyPyPy-3´"

- p53 has a unique DNA binding site that binds to single-stranded DNA ends, allowing into to bind to nonspecific DNA sequences

- p53 can bind to chromatin

Conclusions/action items: p53 binds to DNA and will be present in all affected mutant cells. Thus, we can assume that p53 will be found in urine. This also provided us with two end sequences for p53 to bind to that will give us insight into the peptide sequences for p53.

pRB and cell cycle progression

KARINA BUTTRAM - Dec 14, 2021, 7:30 PM CST

Title: RB and cell cycle progression

Date: 11/4/2021

Content by: Karina Buttram

Present: Karina Buttram

Citation: C. Giacinti, A. Giordano. "RB and cell cycle progression," *Oncogene*, 25,5220-5227, 2006.
Available: https://www.nature.com/articles/1209615. [November 4, 2021].

Goals: further understand what pRb does

Content:

- blocks cells from going into S-phase and entering cell growth

- pRb will bind to chromatin and cause gene inactivation -> mutations

- oncoproteins binding to pRb -> neoplasia for cervical cancer

- Rb proteins have three members: p105, p107, p130 that are called pocket proteins (binding regions for oncoproteins)

- pRb and E2F block cells from moving out of G0 and G1 phases

Conclusions/action items: pRb suppresses tumor growth by blocking cells from getting to the cell growth stage by ensuring that cells do not make it to S-phase of the cell cycle. When oncoproteins bind to pRb, it becomes mutated and can no longer prevent mutated cells from rapidly growing. pRb is able to bind to chromatin and will be present in affected cells, indicating that pRb will be found in urine sediment.

p53 reactivity

KARINA BUTTRAM - Nov 17, 2021, 3:48 PM CST

Title: p53 nuclear protein accumulation correlates with mutations in the p53 gene, tumor grade, and stage in bladder cancer

Date: 11/17/2021

Content by: Karina Buttram

Present: Karina Buttram

Citation: D. Esrig, C. H. Spruck, P. W. Nichols, et al. "p53 nuclear protein accumulation correlates with mutations in the p53 gene, tumor grade, and stage in bladder cancer," *The American Journal of Pathology*, 143(5),1389-1397, 1993. Available:https://pubmed.ncbi.nlm.nih.gov/7901994/. [November 17, 2021].

Goals: understand the reactivity of p53 to determine a possible color changing reactant

Content:

-done in a bladder cancer study

- 84% showed nuclear reactivity, 29% showed immunoreactivity (reactant to particular antigens)

- all mutated p53 showed "high-intensity homogenous immunoreactivity" which is related to the site of the p53 gene mutation

Conclusions/action items: I am still unsure of the chemical reactivity of p53.

Binding Affinity of p53

KARINA BUTTRAM - Nov 21, 2021, 6:42 AM CST

Title: Comparative Binding of p53 to its Promoter and DNA Recognition Elements

Date: 11/21/2021

Content by: Karina Buttram

Present: Karina Buttram

Citation: R. L. Weinberg, D. B. Veprintsev, et al. "Comparative Binding of p53 to its Promoter and DNA Recognition Elements," *Journal of Molecular Biology*, 348(3),589-596, 2005. Available: https://doi.org/10.1016/j.jmb.2005.03.014. [November 21, 2021].

Goals: determine the binding affinity of p53

Content:

- genes involved in apoptosis generally have a lower binding affinity

- this was tested in vitro

- p53 that conform to the typical sequence have a higher binding affinity when binding to tetramers

- binds from the Mdm2 and p21 promoters

- p53 that binds to products that promote apoptosis (in this case E6) have a wide range of binding-affinities

- best binging site: *p21* 5′ site, 4.6(±0.9) nM

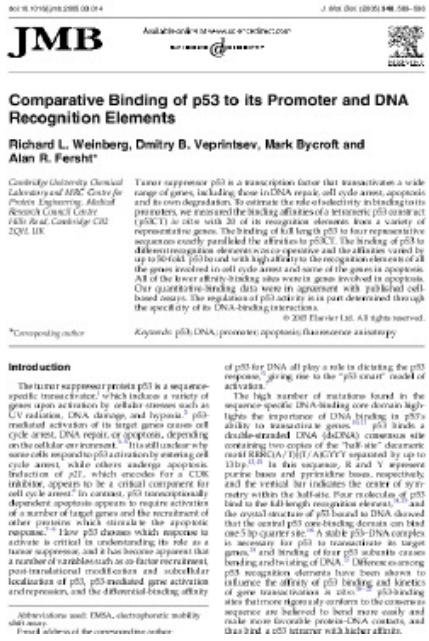
- worst binding site: *P2XM*, 258.8(±51.8) nM

- in general, p53 has a lower binding affinity when binding to pro-apoptosis markers, but it is still co-operative

- higher affinity binding sites for p53 with pro-apoptotic genes: PIDD, PUMA, p53AIP1, and Noxa genes

- "induction of some pro-apoptotic genes occurs only at higher protein concentrations"

Conclusions/action items: It seems that p53 binding to E6 has a lower binding affinity than other p53 binders, but this is reltive to the typical high binding-affinity of p53 to other non-pro-apoptotic genes. It also seems to state that p53 will bind to pro-apoptotic genes if the protein is in higher concentration, possibly indicating that we would need a higher concentration of E6 for the p53 to bind.



JMB

Available online at www.sciencedirect.com

Comparative Binding of p53 to its Promoter and DNA Recognition Elements

Richard L. Weinberg, Dmitry B. Veprintsev, Mark Bycroft and Alan R. Fersht*

Cambridge University Chemical Laboratory and MRC Centre for Protein Engineering, Medical Research Council Centre, Hills Road, Cambridge CB2 2QH, UK

Tumor suppressor p53 is a transcription factor that transactivates a wide range of genes, including those in DNA repair, cell cycle arrest, apoptosis and its own degradation. To estimate the role of selectivity in binding to its promoters, we measured the binding affinities of a tetrameric p53 construct (p53CT) in vitro with 20 of its recognition elements from a variety of representative genes. The binding of full-length p53 to four representative sequences exactly paralleled the affinities to p53CT. The binding of p53 to different recognition elements in co-operative and the affinities varied by up to 50-fold. p53 bound with high affinity to the recognition elements of all the genes involved in cell cycle arrest and some of the genes in apoptosis. All of the lower affinity-binding sites were in genes involved in apoptosis. Our quantitative binding data were in agreement with published cell-based assays. The regulation of p53 activity is in part determined through the specificity of its DNA-binding interactions.

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*Corresponding author

Keywords: p53; DNA; promoter; apoptosis; fluorescence anisotropy

Introduction

The tumor suppressor protein p53 is a sequence-specific transactivator,[1] which induces a variety of genes upon activation by cellular stresses such as UV radiation, DNA damage, and hypoxia.[2] p53-mediated activation of its target genes causes cell cycle arrest, DNA repair, or apoptosis, depending on the cellular environment.[3–5] It is still unclear why some cells respond to p53 activation by entering cell cycle arrest, while others undergo apoptosis. Induction of p21, which encodes for a CDK inhibitor, appears to be a critical component for cell cycle arrest.[6] In contrast, p53 transcriptionally dependent apoptosis appears to require activation of a number of target genes and the recruitment of other proteins which stimulate the apoptotic response.[7–9] How p53 chooses which response to activate is critical to understanding its role as a tumor suppressor, and it has become apparent that a number of variables such as co-factor recruitment, post-translational modification and subcellular localization of p53, p53-mediated gene activation and repression, and the differential binding affinity of p53 for DNA all play a role in dictating the p53 response,[3] giving rise to the "p53 smart" model of activation.

The high number of mutations found in the sequence-specific DNA-binding core domain highlights the importance of DNA binding in p53's ability to transactivate genes.[10,11] p53 binds a double-stranded DNA (dsDNA) consensus site containing two copies of the "half-site" characteristic motif RRRCA/T(T/A)GYYY separated by up to 13 bp.[12,13] In this sequence, R and Y represent purine bases and pyrimidine bases, respectively, and the vertical bar indicates the center of symmetry within the half-site. Four molecules of p53 bind to the full-length recognition element,[14,15] and the crystal structure of p53 bound to DNA showed that the central p53 core-binding domain can bind one 5 bp quarter site.[16] A stable p53–DNA complex is necessary for p53 to transactivate its target genes,[17] and binding of four p53 subunits causes bending and twisting of DNA.[18] Differences among p53 recognition elements have been shown to influence the affinity of p53 binding and kinetics of gene transactivation in vitro.[19–21] p53-binding sites that more rigorously conform to the consensus sequence are believed to bend more easily and make more favorable protein–DNA contacts, and thus bind a p53 tetramer with higher affinity.

Abbreviations used: EMSA, electrophoretic mobility shift assay.

* Email address of the corresponding author:

p53_binding-affinity.pdf(404.1 KB) - download

How latex particles bind to proteins

KARINA BUTTRAM - Dec 01, 2021, 8:19 PM CST

Title: SpheroTechnical Notes #1 - Particles Coating Procedures

Date: 12/1/21

Content by: Karina Buttram

Present: Karina Buttram

Link: https://www.spherotech.com/tech_SpheroTech_Note_1.html

Citation: SpheroTech Inc, "SpheroTechnical Notes #1 - Particles Coating Procedures," *SpheroTech Inc,* 2019. Available: https://www.spherotech.com/tech_SpheroTech_Note_1.html. [December 1, 2021].

Goals: understand how latex particles bind to proteins

Content:

- ligand of choice gets coated in a binding protein: Protein A, Protein G, or Streptavidin

- Protein A, Protein G, or Streptavidin then bind to the latex particles

Conclusions/action items: In theory, we could coat the p53 and pRb proteins in any of these binding proteins to create the sandwich assays.

Home Pregnancy tests

KARINA BUTTRAM - Oct 07, 2021, 5:34 PM CDT

Title: Home pregnancy tests

Date: 10/1/21

Content by: Link: http://www.madehow.com/Volume-4/Home-Pregnancy-Test.html

Citation: "Home pregnancy test," *How products are made.* Internet: http://www.madehow.com/Volume-4/Home-Pregnancy-Test.html. [Oct. 1, 2021].

Present: Karina Buttram

Goals: learn what materials are used to make a pregnancy test

Content:

The test strip

- the strip on the end of a pregnancy test that collects the sample is an immunoassay strip

-strips are formed from compressing fibers and coating them in reactive antibodies to form pads

-the pads are super absorbent

-pads first coated in latex, then assay agent, then up to four antibodies coated

-the immunoassay strip is connected to the absorbent strip that is exposed, as the immunoassay strip cannot get urine directly on it (absorbent pad absorbs the urine and carries it into contact with the immunoassay strip where the reaction occurs to indicate the results of the test)

The plastic part

-made from plastic that has a mechanism on the end to hold the immunoassay strip in place

-clear window allows user to see the results, but protects the immunoassay strip from getting urine directly on it

Packaging

-packaged with a silica packet inside to absorb moisture and help prolong shelf life

Conclusions/action items: This has helped me get an idea for the types of material we will need. We will need immunoassay strips coated in antibodies that can detect cervical cancer and present it on the test strip. We will also need absorbent pads for the end of the testing device and we can 3D print the plastic portion of the device.

Pap Smear

KARINA BUTTRAM - Oct 26, 2021, 3:13 PM CDT

Title: Pap Smear

Date: 9/18/21

Content by: Karina Buttram

Link: https://www.mayoclinic.org/tests-procedures/pap-smear/about/pac-20394841

Citation: Mayo Clinic Staff. "Pap smear," *Mayo Clinic.* Internet: https://www.mayoclinic.org/tests-procedures/pap-smear/about/pac-20394841. [Sept. 18, 2021].

Present: Karina Buttram

Goals: understand exactly what pap smears look for in order to get a better understanding of markers for cervical cancer

Content:

- usually performed with a pelvic exam or HPV test

- tests typically performed every 3 years, looks for any abnormal cells

-high risk patients will be tests more frequently, high risk factors: diagnosis of cervical cancer or pap smear showed precancerous cells, exposure to diethylstilbestrol (DES) before birth, HIV, weakened immune system, or a history of smoking

-abnormal cell results

1. atypical squamos of undetermined significance (ASCUS): grow on the surface of a healthy cervix, do not clearly show precancerous cells if abnormal, doctor can examine the cells for HPV but if they don't indicate HPV the abnormal cells aren't a concern

2. squamos intraepithelial lesion: indicated precancerous cells, low grade changes means the cancer shouldn't show up for years, high grade changes means the cancer could show up much sooner

3. atypical glandular cells: glandular cells produce mucus in your cervix, doesn't not always indicate precancerous cells

4. squamos cell cancer or adenocarcinoma cells: almost certain that cancer is present

Conclusions/action items: Pap smear tests are the most common form of testing for cervical cancer, but it is an invasive procedure. Patients are typically tested every three years unless they show high risk factors. There are four types of abnormal cells that show up on a pap smear. Not all of them always indicate cancerous or precancerous cells, but further testing is required for some cells to tell if it is cervical cancer.

Whiteside Paper-Based Diagnostics

KARINA BUTTRAM - Nov 22, 2021, 9:48 PM CST

Title: Diagnostics, Bioanalytics, and other Tools for Global Health

Date: 11/22/2021

Content by: Karina Buttram

Present: Karina Buttram

Citation: https://gmwgroup.harvard.edu/low-cost-diagnostics-and-tools-global-health

Goals: understand some of Whiteside's paper based tests

Content:

- hydrophobic substances on the surface can direct fluids to wick across it

- developed this to essentially do an ELISA on paper with few other resources

Conclusions/action items: We could essentially replicate an ELISA test on our assay strip and use that for showing the positive or negative result.

Whiteside Paper-based MIcrofluidic Devices

KARINA BUTTRAM - Nov 22, 2021, 10:45 PM CST

Title: Simple Telemedicine for Developing Regions: Camera Phones and Paper-Based Microfluidic Devices for Real-Time, Off-Site Diagnosis

Date: 11/22/2021

Content by: Karina Buttram

Present: Karina Buttram

Citation: A. W. Martinez, S. T. Phillips, E. Carriho, et al. "Simple Telemedicine for Developing Regions: Camera Phones and Paper-Based Microfluidic Devices for Real-Time, Off-Site Diagnosis," *Analytic Chemistry*, 80(10),3699-3707, 2008. Available: https://gmwgroup.harvard.edu/files/1018.pdf. [November 22, 2021].

Goals: better understand Whiteside's microfluidic paper design

Content:

- paper uses lateral-flow immunochromatography and filters solutions

- create a pattern on the paper using hydrophobic walls of polymer, guides them to the site where the assay takes place

- they tested with urine samples

- colorimetric assays are ideal

- used chromatography paper because it wicks fluid very well

- urine uses capillary action to move up the paper, making it a very good substance to obtain noninvasively

Conclusions/action items: We should consider using hydrophobic polymer of some kind to guide the urine sample to the wells we create for the control, p53, and pRb.

KARINA BUTTRAM - Nov 22, 2021, 10:18 PM CST

Simple Telemedicine for Developing Regions: Camera Phones and Paper-Based Microfluidic Devices for Real-Time, Off-Site Diagnosis

1018.pdf(817.7 KB) - download

Lateral flow of Clearblue pregnancy tests

KARINA BUTTRAM - Nov 22, 2021, 11:28 PM CST

Title: Lateral flow and Consumer Diagnostic

Date: 11/22/2021

Content by: Karina Buttram

Present: Karina Buttram

Citation: Sarah Tiplady. "Lateral Flow and Consumer Diagnostics," *The Immunoassay Handbook*, 4,533-536, 2013. Available: https://doi.org/10.1016/B978-0-08-097037-0.00036-1. [November 22, 2021].

Goals: understand how Clearblue tests use lateral flow

Content:

- uses two antibodies: one for the positive result and one that is mobile and is bound to the dye to make the control line

- Dye is a blue-colored latex particles sensitive with monoclonal antibodies, coated in rabbit IgG as the control

- control consists of a goat anti-rabbit immunoglobulin antibody, that latex with rabbit IgG become trapped near the goat " causing the blue line to appear

- blue latex particle also used for the control in ovulation tests

Conclusions/action items: We should use this same control technique given that the antibodies the control tests for is in all urine samples. this will indicate that we have a valid urine sample.

KARINA BUTTRAM - Nov 22, 2021, 11:21 PM CST

CHAPTER 7.3

LATERAL FLOW AND CONSUMER DIAGNOSTICS

Sarah Tiplady

PREGNANCY TESTS

3-s2.0-B9780080970370000361-main.pdf(472 KB) - download

The Home Pregnancy Test

KARINA BUTTRAM - Nov 30, 2021, 3:34 PM CST

Title: The Home Pregnancy Test

Date: 11/30/21

Content by: Karina Buttram

Present: Karina Buttram

Citation: S. Johnson, "The home pregnancy test," *100 Years of Human Chorionic Gonadotropin*, 107-121, 2020. Available: https://doi.org/10.1016/B978-0-12-820050-6.00010-2. [November 30, 2021].

Goals: further understand at home pregnancy tests

Content:

- uses two different antibodies on the test strip

- uses a nitrocellulose test strip

- again uses colored latex particle for the antibody coated in dye for the control line

- strip contains reagents such as buffers to normalize the urine to optimize the antibody reaction

Conclusions/action items: This confirmed that most pregnancy tests use blue latex dye for the control line, which I have seen in other articles as well. This article specified what type of immunoassay strip they use and another reagent we might need to consider putting on the test strip.

KARINA BUTTRAM - Nov 30, 2021, 3:26 PM CST

CHAPTER

The home pregnancy test 2.4

Sarah Johnson

One of the most important questions a woman may have in her life is "Am I Pregnant?" Today's home pregnancy test is able to provide her with this answer in a convenient, discrete, and reliable way. Over 60 million packs of pregnancy tests are sold across Europe per annum, illustrating the tremendous importance of the to products to women. But the home pregnancy test in its current form was only possible due to innovative work by scientists at Unipath* who pioneered the development of the lateral flow test.

Lateral flow technology: complex immunochemistry made user-friendly

Laboratory-based immunoassays require skilled personnel, laboratory equipment, a controlled environment, and several hours to achieve a reliable result. So, they are definitely not suitable for use by untrained women at a time of stress in their own home. A new technology was needed to convert a complex laboratory method to something very simple to use and which could still work after being stocked on the shelves of a pharmacy or supermarket for years; this led to the development of the lateral flow test.

Like standard immunoassay tests, lateral flow assays rely on the specific binding of the target, in this case, human chorionic gonadotropin (hCG), by labeled antibodies and the subsequent removal of any unbound antibodies from the test zone. In lateral flow tests, this is accomplished simply by fluid flow through porous membranes. The technology has been widely exploited in other applications where quick results are needed in nonlaboratory environments. Examples include home fertility testing, drugs of abuse and infectious disease testing in both humans and animals. The technology's versatility means that different sample fluids can be used including urine, blood, saliva or even solid materials broken down in buffer, an example being allergen testing in foods [1].

* Unipath products are now made by SPD Swiss Precision Diagnostics GmbH.

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3-s2.0-B9780128200506000102-main.pdf(1.2 MB) - download

Pregnancy testing device patent

KARINA BUTTRAM - Nov 17, 2021, 4:04 PM CST

Title: United States Patent Application Publication: Pregnancy Test Device and Method

Date: 11/17/2021

Content by: Karina Buttram

Present: Karina Buttram

Citation: https://patentimages.storage.googleapis.com/c7/72/7a/652fd28f34973b/US20150094227A1.pdf

Goals: determine what dye is used to create a color change for the control line

Content:

- use "dyes and protein binders"

- "Various labels suitable for use in the present invention include labels which produce signals through either chemical or physical means, such as being optically detectable. Such labels include enzymes and Substrates, chromogens, catalysts, fluorescent compounds, chemiluminescent com pounds, electroactive species, dye molecules, radioactive labels and particle labels."

- dye using metallic particles such as tellurium or selenium

- dye could be fluorescent of contain a quantum dot

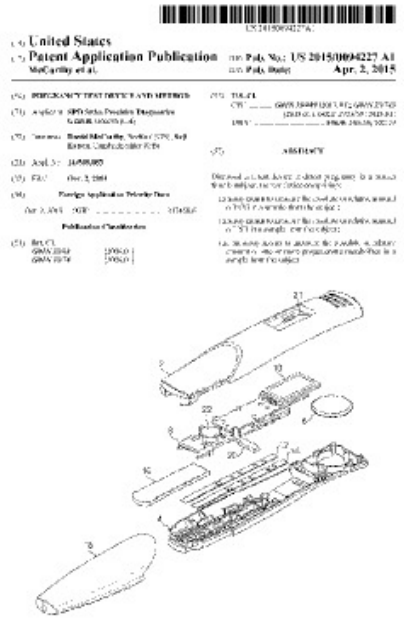
- hydrophobic dyes like Foron Blue SRP (Sandoz) and Resolin Blue BBLS (Bayer)

- synthetic polymer labels (ex. "polystyrene, polyvinyltoluene, polysty rene-acrylic acid and polyacrolein")

- key is that the reagent needs to be hydrophobic

Conclusions/action items: I need to further look into the dyes mentioned in this patent, but we will need to find a color changing reactant that is hydrophobic.

KARINA BUTTRAM - Nov 17, 2021, 7:44 PM CST



US20150094227A1.pdf(2.7 MB) - download

10/3/21 - HPV and Cervical Cancer

Cora Williams - Oct 19, 2021, 11:12 AM CDT

Title: HPV and Cervical Cancer
Date: Oct. 3, 2021
Content by: Cora Williams
Present: Cora Williams
Goals:

- Learn about the connections between HPV and cervical cancer

Content:

- Cervical cancer is the accidental endpoint of persisting infections with certain types of HPV
- HPV 16 is the most important HPV-HR-type; it is linked to approximately 50% of cervical cancers worldwide
- HPV 18 ranks second
- HPV 16 and 18 are associated with two thirds of all cervical cancers as well as subsets of cancers of the vulva, vagina, penis, anus, oropharynx, and skin
- Primary Prevention of Cervical Cancer - HPV Vaccination
    - The global estimates of the protection against cervical cancer of the currently available vaccines in properly vaccinated populations range from 75-80%
- Secondary Prevention of Cervical Cancer - Detection of Precursor Lesions
    - Because of the causal role of HPV in the genesis of cervical cancer, HPV testing has appeared to be a potential screening test since the 1990s
    - One publication found that HPV screening results in a significantly better detection rate of high-grade precursors than Pap smear-based screening
    - As HPV infections are very common below the age of 30 and most of these infections will be self-limiting, HPV screening in this age-group would result in a high rate of meaningless positive results
    - Therefore, the consensus is that HPV screening should start at age 30 with intervals of 5 years for HPV-negative women

Conclusions/action items:

Cervical cancer is the accidental endpoint of persisting infections with certain types of HPV. HPV 16 is the most important HPV-HR type, as it is linked to approximately 50% of cervical cancers worldwide. HPV 18 is the second most important HPV-HR type. These two strains combined are associated with two-thirds of all cervical cancers worldwide. Current prevention strategies for cervical cancer are HPV vaccination and detection of precursor lesions.

The next topic I need to research is the antibody levels in HPV vaccinated women, HPV unvaccinated women, and HPV positive women to see if we could test for HPV antibody levels to determine if someone is HPV positive.

Citation: K. U. Petry, “HPV and cervical cancer,” *Scand. J. Clin. Lab. Invest.*, vol. 74, no. sup244, pp. 59–62, Aug. 2014, doi: 10.3109/00365513.2014.936683.

URL: https://www.tandfonline.com/doi/full/10.3109/00365513.2014.936683

10/13/21 - HPV

Cora Williams - Oct 19, 2021, 11:08 PM CDT

Title: Human Papillomavirus (HPV)

Date: Oct. 13, 2021

Content by: Cora Williams

Present: Cora Williams

Goals:

- Learn what HPV is
- Explore the links between HPV and cervical cancer

Content:

- What is HPV?
  - the most common sexually transmitted infection
  - HPV is usually harmless and goes away by itself, but some types can lead to cancer or genital warts
- The Most Common STD
  - there are more than 200 types of HPV, but only 40 kinds can infect your genital area
  - these kinds of HPV are spread during sexual contact
  - most people who have sex get HPV at some point in their lives
  - most people with HPV have no symptoms and feel totally fine, so they usually don't even know they're infected
  - most HPV infections aren't harmful at all and go away on their own
    - HPV 6 and 11 cause most cases of genital warts
    - HPV 16 and 18 lead to the majority of cancer cases
  - there is no cure for HPV
  - there are vaccines that can help protect you from ever getting certain types of HPV
  - genital warts can be removed by your nurse or doctor
  - high-risk HPV can usually be easily treated before it turns into cancer, which is why regular Pap tests are so important
  - condoms and dental dams can also lower your chances of getting HPV
- How do you get HPV?
  - HPV is easily spread from sexual skin-to-skin contact with someone who has it

Conclusions/action items:

HPV is the most common sexually transmitted infection. HPV is easily spread from sexual skin-to-skin contact with someone who has it. HPV 16 and 18 lead to the majority of cancer cases. High-risk HPV can usually be easily treated before it turns into cancer, which is why regular Pap tests are so important.

Citation: "Human Papillomavirus (HPV)," *Planned Parenthood*. [Online]. Available: https://www.plannedparenthood.org/learn/stds-hiv-safer-sex/hpv. [Accessed: 13-Oct-2021].

URL: https://www.plannedparenthood.org/learn/stds-hiv-safer-sex/hpv

10/18/21 - Urinary HPV Test Could Offer Non-Invasive Alternative to Conventional Smear

Cora Williams - Dec 14, 2021, 7:23 PM CST

Title: Urine HPV Test Could Offer Non-Invasive Alternative to Conventional Smear, Improve Screening Uptake

Date: Oct. 18, 2021

Content by: Cora Williams

Present: Cora Williams

Goals:

- Determine if urine has been used to test for HPV in research studies

Content:

- Compared with cervical samples, urine HPV testing had an overall sensitivity of 87% (the proportion of positives correctly identified) and a specificity of 94% (the proportion of negatives correctly identified)
- Urine testing for "high-risk" HPV types 16 and 18 had an overall sensitivity of 73% and a specificity of 98% compared with cervical samples
- Accuracy increased when "first-void" urine samples were collected (the first urine of the day) compared with random or mid-stream samples, probably because first void urine samples contain higher levels of DNA
- Researchers at the University of Manchester say urine testing for HPV is a promising screening option that deserves further evaluation
- In well resourced health systems, they suggest self sampling "could be used for women who are reluctant to attend for regular cervical screening"
- While in lower income countries that lack infrastructure, "self sampling might even be beneficial and cost effective for all women who are eligible for screening"

Conclusions/action items:

According to this article, one study found urine HPV testing to be a highly sensitive and specific testing method. The overall accuracy was fairly high, and it was increased when first-void urine samples were collected instead of mid-stream or random samples. Researchers say urine testing for HPV is a promising screening option that deserves further evaluation. The article also recognized the potential positive impact a urine HPV test could have on women in both developing and developed countries.

I need to continue researching to determine what biomarkers were used to test for HPV in the urine test.

Citation: “Urine HPV Test Could Offer Non-Invasive Alternative to Conventional Smear, Improve Screening Uptake,” *ScienceDaily*, 17-Sep-2014. [Online]. Available: https://www.sciencedaily.com/releases/2014/09/140917073239.htm. [Accessed: 18-Oct-2021].

URL: https://www.sciencedaily.com/releases/2014/09/140917073239.htm

10/27/21 - Study Shows Promise for Urine-Based Test for HPV-Linked Cervical Cancer

Cora Williams - Dec 14, 2021, 7:27 PM CST

Title: Study Shows Promise for Urine-Based Test for HPV-Linked Cervical Cancer

Date: Oct. 27, 2021

Content by: Cora Williams

Present: Cora Williams

Goals:

- Determine if urine has been used to test for HPV in research studies

Content:

- While they found the urine test showed promise, additional research is needed to improve the test's accuracy
- Already, scientists have developed tests to detect certain oncogenic strains of HPV that can cause cervical cancer, but none have been approved in the United States that test urine specifically
- In their study, researchers compared urine testing using Oncolarity, a U.S. Food and Drug Administration-approved HPV test made by Becton, Dickinson and Company, to the results of a self collected cervical sample, physician exam, and a biopsy for 307 women
- Based on the results of the biopsy, they found that 83 women, or 27 percent, had high-grade precancerous cervical abnormalities
- Urine testing for HPV was able to identify 80 percent of those cases
- In comparison, researchers found testing self-collected cervical samples and physician-collected samples for high-risk HPV strains were somewhat more accurate
- Using those methods, patients and physicians were able to detect 94 percent of high-risk cervical cancer lesions
- Researchers reported that since the urine testing sensitivity levels for detection of high-grade cervical pre-cancer were lower than other forms of screening, work is needed to improve the accuracy of the test

Conclusions/action items:

This article discussed the findings of a study on urine HPV testing. It had many similar results to the previous article I read (urine shows promise, but still needs more work).

I need to continue research to determine what HPV biomarkers are present in urine.

Citation: “Study Shows Promise for Urine-Based Test for HPV-Linked Cervical Cancer,” *UNC School of Medicine*, 07-Feb-2020. [Online]. Available: https://unclineberger.org/news/study-urine-based-test-for-hpv-linked-cervical-cancer/. [Accessed: 27-Oct-2021].

URL: https://unclineberger.org/news/study-urine-based-test-for-hpv-linked-cervical-cancer/

11/3/21 - Detection of HPV E6 Oncoprotein from Urine via a Novel Immunochromatographic Assay

Cora Williams - Dec 14, 2021, 7:29 PM CST

Title: Detection of HPV E6 Oncoprotein from Urine via a Novel Immunochromatographic Assay

Date: Nov. 3, 2021

Content by: Cora Williams

Present: Cora Williams

Goals:

- Determine how this study tested for the HPV E6 Oncoprotein

Content:

- Abstract
- Introduction
- Materials and Methods
    - Study Population
    - Specimen Collection
        - Prior to a pelvic examination, women provided a random urine sample in a 80 mL polypropylene container, and a self-collected vaginal sample was obtained via the Viba-Brush
        - Women then underwent pelvic examination by a gynecologist, and a physician-collected cervical scraping was obtained via the Cervex-Brush Combi
        - Six milliliters of urine were added to a 50mM solution of EDTA and used to perform HPV DNA testing
        - The remaining urine was used to perform the Onco***E6***™ Cervical Test without solution of EDTA
    - HPV Tests
        - An aliquot of six milliliters of urine added to a 50mM solution of EDTA was used to perform the HPV DNA testing according to the Cobas® 4800 HPV standard protocol
        - Urine specimens were shaken up before aliquots of 7.5mL, 15mL or 30mL were removed and centrifuged
        - The resulting pellet was also suspended in 930µL of Rinse Solution and then transferred to the test tube
        - The test was performed according to the manufacturer's instructions except for the extraction step: upon communication with the manufacturer, volumes for the Lysis Solution and the Conditioning Solution were deceased by 50% with regard to the regular protocol; 416µL and 39µL were used
    - Statistical Analysis
- Results
    - High-Risk HPV DNA Results (Cobas 4800 HPV Test)
    - HPV 16/18 E6 Results (OncoE6 Cervical Test)
        - In urine samples, HPV16-E6 was detected in 22 specimens and HPV18-E6 in 4
        - Using the cervical sample collected by the physician as the reference, the positivity rate of HPV16/18-E6 test was significantly higher than the vaginal self-collection (respectively 30.6% vs. 20.2%; $p<0.01$) and the urine (respectively 30.6% vs. 21.0%, $p<0.01$)
        - Regarding the positivity of HPV16/18-E6 specifically in urine it was significantly higher in the group of women with invasive carcinoma compared

to the other groups (<CIN2 and CIN2/3)
        - No HPV16/18-E6 positive result was obtained in the CIN2 group (0/9) in the three type of specimens
        - In CIN3 group, the HPV16/18-E6 was positive in 26.9% (7/26) of the cervical samples and in 3.8% (1/26) of both, vaginal and urine samples
        - There was no significant difference in urine HPV16/18-E6 positivity between the group of women without cervical injury and those diagnosed with high-grade precursor lesion
    - Comparative Analysis between the HPV16/18-E6 test and the HPV16/18-DNA test
        - The HPV16/18-E6 positivity rate was significantly lower (<0.01) than the HPV DNA positivity rate for HPV types 16 and 18 when the analysis was stratified by specimen type (cervical, vaginal and urine)
        - Comparison of the HPV16/18-E6 test with the HPV DNA test showed moderate agreement in the urine and vaginal samples (self-collection), and moderate to strong agreement in the cervical sample (physician-collection)
    - Clinical accuracy of HPV16/18-E6 and HPV-DNA tests
        - The HPV16/18-E6 HPV test had a significantly lower sensitivity rate than the HPV-DNA test for both CIN2 and CIN3 detection, regardless of the type of specimen (cervical, vaginal or urine)
        - On the other hand, it presented higher specificity rate than the HPV-DNA test
    - Discussion
    - Conclusions

Conclusions/action items:

This article discussed the findings of a study on urine HPV testing. It had many similar results to the previous article I read (urine shows promise, but still needs more work).

I need to continue research to determine what reagents will react with E6/E7 oncoproteins.

Citation: C. M. Oliveira, L. W. Musselwhite, N. de Paula Pantano, F. L. Vazquez, J. S. Smith, J. Schweizer, M. Belmares, J. C. Possati-Resende, M. de Vieira, A. Longatto-Filho, and J. H. Fregnani, “Detection of HPV E6 Oncoprotein from Urine via a Novel Immunochromatographic Assay,” *PLOS ONE*, vol. 15, no. 4, 2020.

URL: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0232105

11/3/21 - A Quantitative LumiFlo Assay to Test Inhibitory Compounds Blocking p53 Degradation Induced by HPV

Cora Williams - Dec 14, 2021, 7:31 PM CST

Title: A Quantitative *LumiFluo* Assay to Test Inhibitory Compounds Blocking p53 Degradation Induced by Human Papillomavirus Oncoprotein E6 in Living Cells

Date: Nov. 3, 2021

Content by: Cora Williams

Present: Cora Williams

Goals:

- Determine what part of the DNA E6 oncoproteins attack

Content:

- E6 is a very small cysteine-rich protein whose physiological role is to keep the infected cell in an S-phase-like state, cooperating with E7 for efficient cellular hijacking
- High-risk E6 proteins target p53 for proteasome-mediated degradation, while E7 can inhibit the activity of pRb, thus forcing the cell to continuously proliferate and accumulate somatic mutations
- E6 possesses a multifaceted inhibitory activity against p53, acting directly against the protein as well as against other cellular factors that normally lead to the activation of p53, such as p300 and ADA3
- In addition, E6 can bind several other cellular proteins to induce their degradation through the cellular proteasome machinery, such as procaspase 8, Bak, Scribble and MAGI-1
- The viral E6 oncogene undergoes massive splicing events, producing several truncated isoforms in addition to the full-length protein, but only the latter mediates the degradation of p53
- Mechanistically, full-length high-risk E6 proteins can efficiently induce p53 degradation through the direct association with both p53 and the cellular ubiquitin ligase E6AP, to form a trimeric complex that leads to p53 ubiquitination and degradation

Conclusions/action items:

This article discussed the proteins that E6/E7 oncoproteins bind to. It also described the exact functions of E6 and E7.

I need to continue research to determine what peptide sequences E6/E7 oncoproteins bind to within the proteins discussed above.

Citation: L. Messa, M. Celegato, C. Bertagnin, B. Mercorelli, G. Nannetti, G. Palù, and A. Loregian, “A quantitative LumiFluo assay to test inhibitory compounds blocking p53 degradation induced by human papillomavirus oncoprotein E6 in living cells,” *Nature News*, 16-Apr-2018. [Online]. Available: https://www.nature.com/articles/s41598-018-24470-4#citeas. [Accessed: 03-Nov-2021].

URL: https://www.nature.com/articles/s41598-018-24470-4

12/1/21 - Development and Validation of a Multiplex Immunoassay for the Simultaneous Quantification of Type-Specific IgA Antibodies to E6/E7 Oncoproteins

Cora Williams - Dec 14, 2021, 7:34 PM CST

Title: Development and Validation of a Multiplex Immunoassay for the Simultaneous Quantification of Type-Specific IgG Antibodies to E6/E7 Oncoproteins of HPV16 and HPV18

Date: Dec. 1, 2021

Content by: Cora Williams

Present: Cora Williams

Goals:

- Determine what concentrations of E6/E7 antibodies are detectable

Content:

- This report describes a multiplex assay leveraging the spot printing of the MSD electrochemiluminescent technology based on the fusion tagging of HPV oncoproteins to simultaneously detect antibody concentrations to HPV16 E6 and E7 as well as HPV18 E6 and E7 in a high-throughput manner with a small amount of sample needed to conduct the assay (500-fold dilution of sample in a 50 µL volume).
- The synthesis of these novel oncoproteins allowed us to detect a wide range of antibody concentrations to HPV proteins in human serum, HPV+ cervical cancer serum, and vaccinated subjects to HPV oncoproteins with precision, reproducibility, and some degree of cross-reacticity between HPV16 and 18 E6 antibodies
- While we hypothesized that HPV+ subjects would have greater antibody concentrations, we found a modest antibody concentration increase in HPV+ subjects to normal healthy donors.
    - This may be attributed to the broad prevalence of HPV16 and 18 positivity in the general population due to the number of sexual partners lending itself the need to identify a true negative population to set serostatus cutoff values from individuals with less than 1 sexual partner.
    - Furthermore, we found low antibody concentrations in our pediatric donor cohort which may be attributed to material antibodies as these children were between ages 1–5 day
- In setting a serostatus (the state of either having or not having detectable antibodies against a specific antigen), as measured by a blood test) cutoff value for these HPV antibodies, we would have increased confidence in identifying pharmacodynamic attributes of patients on current HPV vaccine trials targeting HPV16 and 18 oncoproteins E6 and E7, however we cannot rule out that the observed signal in non-HPV+ populations may be attributed to some degree of a lack of specificity

Table 2

Estimated assay upper limit of quantitation (ULOQ) and percent recovery for type-specific anti-HPV concentrations from reference serum.

Antigen	Expected Concentration (AU/mL)	Observed Concentration (AU/mL)	Percent Recovery	Intra-plate %CV	Inter-plate %CV	Estimated ULOQ (AU/mL)
HPV16 E6	30	30.8	103%	4.3	6.2	25.5
	25	26.5	106%	3.4	5.8	
	20.8	21.9	105%	1.8	3.8	
	17.4	18.5	106%	3.8	7.4	
HPV16 E7	5	5.3	106%	4	8.7	4.25
	4.2	4.5	107%	3.5	6.6	
	3.5	3.8	109%	1.4	9	
	2.9	3.2	110%	6.5	13.1	
HPV18 E6	50	48.9	98%	4	6.3	42.5
	41.7	43.4	104%	4.8	6.4	
	34.7	36.6	105%	3.5	4	
	28.9	30.2	104%	5.4	7.4	
HPV18 E7	20	19.8	99%	6.3	6.3	17
	16.7	16.9	101%	4.4	5.3	
	13.9	14.1	101%	4.4	4.8	
	11.6	11.3	97%	10.4	12.1	

Table 3

Estimated assay lower limit of quantitation and percent recovery for type-specific anti-HPV concentrations of the reference serum.

	Series 1					Series 2					Estimated LLOQ	
Antigen	Expected Concentration (AU/mL)	Observed Concentration (AU/mL)	Percent Recovery	Intra-plate %CV	Inter-plate %CV	Expected Concentration (AU/mL)	Observed Concentration (AU/mL)	Percent Recovery	Intra-plate %CV	Inter-plate %CV	Signal	Concentration (AU/mL)
HPV16 E6	0.600	0.6953	116%	7.1	16.8	0.120	0.0973	81%	4.3	15.3	1000	0.0681
	0.400	0.4141	104%	4.8	16.8	0.080	0.0729	91%	3.7	11.3		
	0.267	0.2765	104%	3.2	12.9	0.053	0.0509	95%	2.7	19.2		
	0.178	0.1623	91%	4.9	25.9	0.036	0.0305	86%	3.6	22.6		
	0.119	0.1112	94%	7.3	19.6	0.024	0.0187	79%	16.0	36.1		
	0.079	0.0762	96%	5.0	30.5	0.016	0.0131	83%	21.6	33.7		
	0.053	0.0474	90%	11.5	36.8	0.011	0.0087	83%	8.0	30.1		
	0.035	0.0297	85%	3.3	32.6	0.007	0.0051	73%	31.1	46.7		
HPV16 E7	0.100	0.1182	118%	7.0	11.4	0.020	0.0177	89%	22.9	24.0	600	0.0457
	0.067	0.0672	101%	4.2	18.5	0.013	0.0135	101%	24.4	24.6		
	0.044	0.0443	100%	7.6	18.3	0.009	0.0095	107%	40.1	43.4		
	0.030	0.0266	90%	9.8	35.1	0.006	0.0059	100%	42.7	44.6		
	0.020	0.0201	102%	11.0	19.7	0.004	NE	NE	56.8	56.8		
	0.013	0.0143	109%	15.6	35.6	0.003	NE	NE	64.8	64.8		
	0.009	0.0074	84%	22.5	36.8	0.002	NE	NE	39.9	42.8		
	0.006	0.0047	80%	NE	38.6	0.001	NE	NE	69.3	67.2		
HPV18 E6	1.000	1.1673	117%	7.6	16.6	0.200	0.1511	76%	20.0	12.5	2500	0.1265
	0.667	0.6814	102%	5.5	18.8	0.133	0.1145	86%	11.7	11.0		
	0.444	0.4515	102%	2.8	15.0	0.089	0.0813	91%	18.9	17.4		

HPV18 E6	1.000	1.1673	117%	7.6	16.6	0.200	0.1511	76%	20.0	12.5	2500	0.1265
	0.667	0.6814	102%	5.5	18.8	0.133	0.1145	86%	11.7	11.0		
	0.444	0.4515	102%	2.8	15.0	0.089	0.0813	91%	18.9	17.4		
	0.296	0.2649	89%	4.7	29.5	0.059	0.0471	79%	18.2	19.5		
	0.198	0.1912	97%	5.9	25.0	0.040	0.0301	76%	24.0	26.1		
	0.132	0.1281	97%	7.1	35.5	0.026	0.0221	84%	33.6	36.8		
	0.088	0.0794	90%	9.0	36.0	0.018	0.0144	82%	37.7	24.0		
	0.059	0.0507	87%	5.7	32.6	0.012	0.0087	74%	32.2	33.5		
HPV18 E7	0.400	0.4964	124%	10.3	12.4	0.080	0.0642	80%	21.6	15.9	1000	0.0561
	0.267	0.2732	102%	5.4	13.0	0.053	0.0478	90%	17.2	18.6		
	0.178	0.1818	102%	2.7	12.0	0.036	0.0327	92%	19.5	20.0		
	0.119	0.1056	89%	7.5	27.4	0.024	0.0174	73%	35.2	37.9		
	0.079	0.0782	99%	5.5	19.4	0.016	0.0125	79%	40.4	42.1		
	0.053	0.0494	94%	5.7	31.4	0.011	0.0082	78%	41.0	42.6		
	0.035	0.031	88%	8.9	32.0	0.007	0.0055	78%	48.7	51.4		
	0.023	0.0197	84%	7.1	25.5	0.005	0.0033	70%	40.3	33.5		

Conclusions/action items:

This article discussed the concentrations of E6/E7 antibodies in blood that are required for dectection. It appears that the researchers were able to detect very small amounts of E6/E7 antibodies in blood serum.

Citation: H. Layman, K. W. Rickert, S. Wilson, A. A. Aksyuk, J. M. Dunty, D. Natrakul, N. Swaminathan, and C. J. DelNagro, “Development and validation of a multiplex immunoassay for the simultaneous quantification of type-specific IGG antibodies to E6/E7 oncoproteins of HPV16 and HPV18,” *PLOS ONE*, vol. 15, no. 3, 2020.

URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7098588/

12/1/21 - Guide to Labeling Your Primary Antibody

Cora Williams - Dec 14, 2021, 7:36 PM CST

Title: Guide to Labeling Your Primary Antibody

Date: Dec. 1, 2021

Content by: Cora Williams

Present: Cora Williams

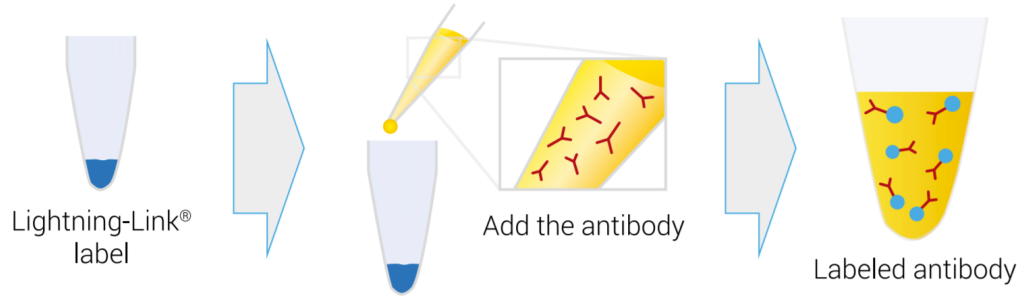
Goals:

- Determine how to coat our antibodies in dye

Content:

- The Lightning-Link process is summarized in Figure 4 (shown below)
- The antibody to be labeled is added to a vial of lyophilized mixture containing the particular label of interest; over 40 labels are available in this format including dyes, proteins and enzymes.
- Dissolution of the vial contents activates the chemicals that mediate the antibody labeling reaction
- The byproducts of the reaction are completely benign and antibody recovery is 100%
- Furthermore the hands-on time is just thirty seconds, and this is true whatever the scale of reaction (10µg-100mg range).

Figure 4. Lightning-Link antibody labeling process



Appendix: Summary of covalent technologies used to attach labels to antibodies

	Lightning-Link	Gold & Latex (Innova)	NHS ester	Isothio-cyanate	Carbodi-imide	Two-tag	Periodate
Avoids tagging of antibody	Yes	Yes	Yes	Yes	Yes	No	Yes
Avoids post-conjugation separations	Yes	Yes[1]	No	No	No	Yes	Yes[2]
Used to attach enzymes	Yes	n/a	No	No	No	Yes	Yes[3]
Used to attach dyes or small molecules	Yes	n/a	Yes	Yes	No	No	No
Scalability	Easy	Easy	Hard	Hard	Easy	Very difficult	Hard[4]
Hands-on time 30 seconds	30 seconds	1-3 min	>15 min	>15min	>15min	>60 min	>15 min
10µg scale possible?	Yes	Yes (also 1µg)	No	No	No	No	Yes[2]
Typical antibody yield	100%	100%[1]	50-80%	50-80%	50-80%	20-50%	70-80%
Other comments	One step, no losses	One step, no losses	NHS esters unstable	High pH needed	Used with particle labels	Complex multi-step process	Chemical hazards

Conclusions/action items:

This article discussed the different methods for labeling primary antibodies. After reading the article, it appears that the best way to coat antibodies in dye is the Lightning-Link method (shown in the diagram above). This is a simple method with fantastic labeled antibody yield.

Citation: "Guide to Labeling Your Primary Antibody," *Innova Biosciences*. [Online]. Available: https://www.bidmc.org/-/media/files/beth-israel-org/research/core-facilities/flow-cytometry-core/guide-to-labeling-your-primary-antibody-2017.pdf. [Accessed: 01-Dec-2021].

URL: https://www.bidmc.org/-/media/files/beth-israel-org/research/core-facilities/flow-cytometry-core/guide-to-labeling-your-primary-antibody-2017.pdf

Cora Williams - Dec 01, 2021, 7:47 PM CST

Innova Biosciences Guide

Guide-to-labeling-your-primary-antibody-2017.pdf(1.2 MB) - download

12/1/21 - Paper-Based Microfluidic Point-of-Care Diagnostic Devices

Cora Williams - Dec 14, 2021, 7:38 PM CST

Title: Paper-Based Microfluidic Point-of-Care Diagnostic Devices

Date: Dec. 1, 2021

Content by: Cora Williams

Present: Cora Williams

Goals:

- Determine how our antibodies will be immobilized on the test strip

Content:

- Next, the sample and conjugate couples migrate to the reaction matrix, which consists of two bands where antibodies have previously been immobilised.
- These two bands act as a capture mechanism for the conjugate along with the analyte.
- Nitrocellulose has been the material of choice as the reaction matrix in lateral flow assays for the last two decades
- The attractiveness of nitrocellulose relates to its ability to bind irreversibly and hydrophobically to proteins by absorption.

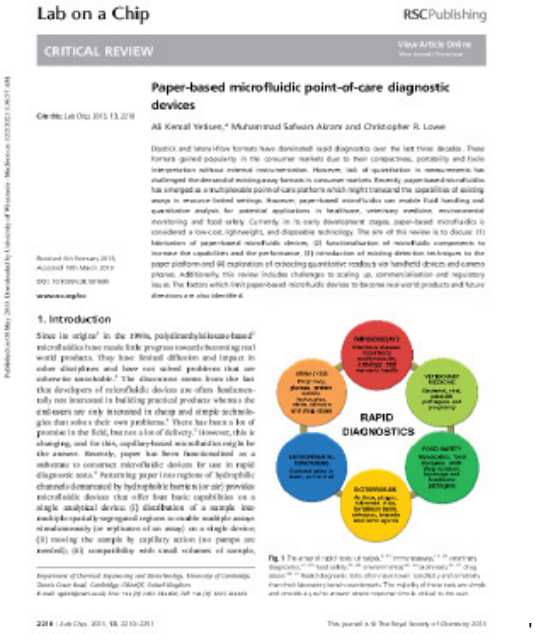
Conclusions/action items:

This article discussed how lateral flow assay tests work. After reading the article, it appears that the best way to immobilize our antibodies on the test strip is to "paint" an antibody strip onto a nitrocellulose strip, which will bind irreversibly to the antibodies and prevent them from moving.

Citation: A. K. Yetisen, M. S. Akram, and C. R. Lowe, “Paper-Based Microfluidic Point-of-Care Diagnostic Devices,” *Lab on a Chip*, vol. 13, no. 12, p. 2210, 2013.

URL: https://pubs.rsc.org/en/content/articlepdf/2013/lc/c3lc50169h

Cora Williams - Dec 01, 2021, 8:01 PM CST



Lab on a Chip

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CRITICAL REVIEW

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Paper-based microfluidic point-of-care diagnostic devices

Ali Kemal Yetisen,* Muhammad Safwan Akram and Christopher R. Lowe

1. Introduction

RAPID DIAGNOSTICS

Paper-Based_Microfluidic_Point_of_Care_Diagnostic_Devices.pdf(3.1 MB) - download

11/3/21 - OncoE6 Cervical Test

Cora Williams - Dec 12, 2021, 4:15 PM CST

Title: OncoE6 Cervical Test

Date: Nov. 3, 2021

Content by: Cora Williams

Present: Cora Williams

Goals:

- Determine how the OncoE6 Cervical Test works
- Determine how the OncoE6 Cervical Test compares to our design

Content:

- HPV and Cervical Cancer
  - it is now known that HPV produces two oncoproteins, E6 and E7, without which cancer does not occur
  - detection of HPV DNA or RNA simply identifies infection
  - detection of the E6 and/or E7 oncoproteins, however, provides a means of identifying the transition from infection toward cancer
- OncoE6 Cervical Test
  - the OncoE6 Cervical Test is a rapid and easy-to-use lateral flow assay based on the detection of E6 oncoproteins
  - the OncoE6 Cervical Test is available in the US, as a service through our CLIA-certified laboratory
  - the Onco***E6***™ Cervical test demonstrates outstanding clinical performance with high specificity and high positive predictive value and thus can be used to triage patients with high risk HPV and other abnormal screening results to avoid unnecessary treatment procedures
  - this qualitative test is used to analyze cells extracted from cervical cytology swab specimens
  - the assay is based upon the capture and detection of E6 oncoproteins from high risk HPV types 16 and 18 using highly specific monoclonal antibodies (mAbs) in a lateral-flow (LF) assay format
  - this test detects down to a thousand abnormal cells with a simple line read by eye
  - the test is room temperature stable and requires no complex equipment

Conclusions/action items:

The OncoE6 Cervical Test is a cervical cancer test that is already on the market in both the US and Europe. It accomplishes many of the goals we set for ourselves. As a result, much of Arbor Vita's research and studies will be applicable to our design.

I need to research the monoclonal antibodies used in the OncoE6 Cervical test and processes to lyse cells.

Citation: “OncoE6 Cervical Test,” *Arbor Vita Corporation*, 25-May-2020. [Online]. Available: https://www.arborvita.com/oncoe6/. [Accessed: 03-Nov-2021].

URL: https://www.arborvita.com/oncoe6/

11/17/21 - Performance of OncoE6 Cervical Test

Cora Williams - Dec 14, 2021, 7:40 PM CST

Title: Performance of OncoE6 Cervical Test in Detecting Cervical Precancer Lesions in HIV-Positive Women Attending an HIV Clinic in Bujumbura, Burundi: A Cross-Sectional Study

Date: Nov. 17, 2021

Content by: Cora Williams

Present: Cora Williams

Goals:

- Determine how the well the OncoE6 Cervical Test test for cervical cancer

Content:

- In a clinical performance of different screening tests at CIN2+ and CIN3+ thresholds among HIV-infected women in Burundi, OncoE6 had a 2% false positive rate at the CIN2+ and CIN3+ disease-positive thresholds.
- In a clinical performance of the algorithm HPV+ test followed by OncoE6 or VIA test, OncoE6 had a false positive rate of approximately 5% at both the CIN2+ and CIN3+ thresholds.
- This study does not recommend the OncoE6 Cervical Test for primary screening because of its low sensitivity and poor performance in identifying CIN2+ lesions
- The researchers highlighted the need for an OncoE6 test to incorporate a wide range of HR-HPV strains, which would result in a good test performance for primary cervical cancer screening with less stringent equipment and personnel requirements.

Conclusions/action items:

The OncoE6 Cervical Test is a cervical cancer test that is already on the market in both the US and Europe. It accomplishes many of the goals we set for ourselves. However, one study did not recommend the test for primary screening because of its low sensitivity and poor performance in identifying CIN2+ lesions. The researchers highlighted the need for an OncoE6 test to incorporate a wide range of HR-HPV strains, which would result in a good test performance for primary cervical cancer screening with less stringent equipment and personnel requirements.

I need to research what other cancers E6 and E7 oncoproteins are found in.

Citation: Z. Ndizeye, S. Menon, J.-P. Van Geertruyden, C. Sauvaget, Y. Jacquemyn, J.-P. Bogers, I. Benoy, and D. Vanden Broeck, “Performance of oncoe6tmcervical test in detecting cervical precancer lesions in HIV-positive women attending an HIV clinic in Bujumbura, Burundi: A cross-sectional study,” *BMJ Open*, vol. 9, no. 9, 2019.

URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6731839/

9/23/21 - Average Cost of Pap Smear

Cora Williams - Oct 19, 2021, 11:22 AM CDT

Title: Average Cost of a Pap Smear in the United States

Date: Sept. 23, 2021

Content by: Cora Williams

Present: Cora Williams

Goals:

- Determine the average cost of a Pap smear

Content:

- In the United States, the average cost of a pelvic exam and a Pap smear is $331 without insurance.

Conclusions/action items:

Pap smears are rarely preformed without a pelvic exam. As a result, the average cost of a pelvic exam and Pap smear is $331 without insurance in the United States. The majority of the cost is from having a medical professional collect the sample and from having to send the sample out to a lab for testing. If we can eliminate these expenses, the overall cost of our testing device should be significantly lower.

The next topics I need to research are the average household income in Ethiopia and how the Ethiopian healthcare system operates.

Citation: A. Corso, “How much does a Pap Smear cost without insurance in 2021?,” *Mira*, 20-Aug-2021. [Online]. Available: https://www.talktomira.com/post/how-much-does-a-pap-smear-cost. [Accessed: 23-Sep-2021].

URL: https://www.talktomira.com/post/how-much-does-a-pap-smear-cost

9/23/21 - Average Household Income in Ethiopia

Cora Williams - Oct 19, 2021, 11:26 AM CDT

Title: Average Household Income in Ethiopia

Date: Sept. 23, 2021

Content by: Cora Williams

Present: Cora Williams

Goals:

- Determine the average household income in Ethiopia

Content:

- The average income for a household in Ethiopia is US $354.

Conclusions/action items:

The average income for a household in Ethiopia is US $354. Considering the average cost of a Pap smear in the United States without insurance, it is obvious why our client wants to keep the cost of our test as low as possible.

The next topic I need to research is how the Ethiopian healthcare system operates.

Citation: R. Bluffstone, M. Yesuf, B. Bushie, and D. Damite, “Rural Livelihoods, Poverty, and the Millennium Development Goals,” *Environ. Dev.*, no. June 2008, Jun. 2008, Accessed: Sep. 23, 2021. [Online]. Available: https://media.rff.org/documents/EfD-DP-08-07.pdf

URL: https://media.rff.org/documents/EfD-DP-08-07.pdf

9/18/2021 - Exosomal let-7d-3p and miR-30d-5p as diagnostic biomarkers for non-invasive screening of cervical cancer and its precursors

Josephine HALL (jrhall3@wisc.edu) - Sep 18, 2021, 7:03 PM CDT

Title: Exosomal let-7d-3p and miR-30d-5p as diagnostic biomarkers for non-invasive screening of cervical cancer and its precursors

Date: 9/18/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Further understand the biomarkers suggested by the client for use in the detection of cervical cancer

Content:

Found: Pubmed data base, searched 'Exosomal let-7d-3p and miR-30d-5p'

Citation:

> M. Zheng, L. Hou, Y. Ma, L. Zhou, F. Wang, B. Cheng, W. Wang, B. Lu, P. Liu, W. Lu, and Y. Lu, “Exosomal let-7D-3P and Mir-30d-5p as diagnostic biomarkers for non-invasive screening of cervical cancer and its precursors,” *Molecular Cancer*, vol. 18, no. 1, 2019.

Notes:

- Cervical cancer is second leading cause of cancer in women from 20-39 years old
- Pap smear and TCT (Thinprep Cytological Test) used as screening methods
  - (These tests not commonly used in China - especially rural regions
- Personal beliefs and cultural factors prevent women from using theses screening tests

Exosomes:

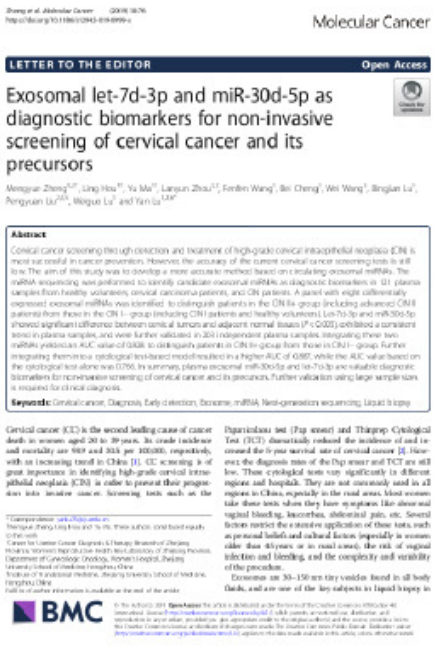
- 30-150 nm vesicles in all body fluids
- deliver DNA fragments, mRNA, proteins, lipids
- exosomal miRNA are stable and non-degradable

Study:

- 121 plasma samples from healthy volunteers, cervical cancer patients, and precancerous patients
- miRNA sequencing was performed
- current cytology test have relatively low accuracy when compared to cervical biopsy
- CIN1 patients have reversible diagnosis and do not have to be medicated or surgically operated on
- CIN1- group was CIN 1 patients and healthy individuals
- CIN II+ group is combination of CIN II-III patients and CC patients that need treatment
- average age 50+-24 years
- CIN I- samples used as reference
- CIN II-III and CC shared common miRNA expression profiles
- 37 DEmiRs identified between CIN I- and CIN II+
- picked 8 strongest predictors from the 37
- no significant differences in expression of miRNAs in the best panel (8) between different HPV types
- pathways studied on the miRNA targets - top targeted pathway was viral carcinogenesis (consistent with CC and caused by HPV)
- miR-30d-5p regulate genes involved in many of the significant pathways studied
- let-7d-3p and miR-30d-5p showed significant differences in expression between cancerous and pap-carcinoma tissue
- expression levels of let-7d-3p and miR-30d-5p were significantly decreased in CIN II+ group compared to CIN I- group
- blood test in this experiment

Conclusions/action items: This article provided an improved understanding of biomarkers that are useful in the detection of cervical cancer. Further research will need to be completed to fully understand the topic. Will look into the Pap smear and TCT test to understand other cervical cancer screening tests

Josephine HALL (jrhall3@wisc.edu) - Sep 18, 2021, 6:20 PM CDT



Molecular Cancer

LETTER TO THE EDITOR Open Access

Exosomal let-7d-3p and miR-30d-5p as diagnostic biomarkers for non-invasive screening of cervical cancer and its precursors

Mengyue Zheng[†], Ling Hou[†], Yu Ma[†], Lanyun Zhou[†], Fenfen Wang[*], Bei Cheng[*], Wei Wang[*], Binglian Lu[*], Pengyuan Liu[*], Weiguo Lu[*] and Yan Li[*]

Abstract

Cervical cancer screening through detection and treatment of high-grade cervical intraepithelial neoplasia (CIN) is most successful in cancer prevention. However, the accuracy of the current cervical cancer screening tests is still low. The aim of this study was to develop a more accurate method based on circulating exosomal miRNAs. The miRNA sequencing was performed to identify candidate exosomal miRNAs as diagnostic biomarkers in 121 plasma samples from healthy volunteers, cervical carcinoma patients, and CIN patients. A panel with eight differentially expressed exosomal miRNAs was identified to distinguish patients in the CIN III+ group (including advanced CIN III patients) from those in the CIN I− group (including CIN I patients and healthy volunteers). Let-7d-3p and miR-30d-5p showed significant difference between cervical tumors and adjacent normal tissues ($P < 0.001$), exhibited a consistent trend in plasma samples, and were further validated in 204 independent plasma samples. Integrating these two miRNAs yielded an AUC value of 0.868 to distinguish patients in CIN III+ group from those in CIN I− group. Further integrating them into a cytological test-based model resulted in a higher AUC of 0.897, while the AUC value based on the cytological test alone was 0.766. In summary, plasma exosomal miR-30d-5p and let-7d-3p are valuable diagnostic biomarkers for non-invasive screening of cervical cancer and its precursors. Further validation using large sample sizes is required for clinical diagnosis.

Keywords: Cervical cancer, Diagnosis, Early detection, Exosome, miRNA, Next-generation sequencing, Liquid biopsy

Cervical cancer (CC) is the second leading cause of cancer death in women aged 20 to 39 years. Its crude incidence and mortality are 9.9/1 and 3.1/1 per 100,000, respectively, with an increasing trend in China [1]. CC screening is of great importance in identifying high-grade cervical intraepithelial neoplasia (CIN) in order to prevent their progression into invasive cancer. Screening tests such as the Papanicolaou test (Pap smear) and Thinprep Cytological Test (TCT) dramatically reduced the incidence of and increased the 5-year survival rate of cervical cancer [2]. However, the diagnosis rates of the Pap smear and TCT are still low. These cytological tests vary significantly in different regions and hospitals. They are not commonly used in all regions in China, especially in the rural areas. Most women take these tests when they have symptoms like abnormal vaginal bleeding, leucorrhea, abdominal pain, etc. Several factors restrict the extensive application of these tests, such as personal beliefs and cultural factors (especially in women older than 45 years or in rural areas), the risk of vaginal infection and bleeding, and the complexity and variability of the procedure.

Exosomes are 30–150 nm tiny vesicles found in all body fluids, and are one of the key subjects in liquid biopsy in

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†Mengyue Zheng, Ling Hou and Yu Ma, these authors contributed equally to their work.
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Exosomal_let-7D-3P_and_Mir-30d-5p_as_diagnostic_biomarkers_for_non-invasive_screening_of_cervical_cancer_and_its_precursors.pdf(1.5 MB) - download

9/26/2021 - Non-invasive Assessment of Vaccine-Induced HPV Antibodies via First-Void Urine

Josephine HALL (jrhall3@wisc.edu) - Sep 26, 2021, 10:25 PM CDT

Title: Non-invasive Assessment of Vaccine-Induced HPV Antibodies via First-Void Urine

Date: 9/26/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Investigate the use of urine as a method of non-invasive HPV testing

Content:

Pubmed data base

searched "HPV urine tests"

Citation:

> J. Pattyn, S. Van Keer, L. Téblick, P. Van Damme, and A. Vorsters, “Non-invasive assessment of vaccine-induced HPV antibodies via first-void urine,” *Frontiers in Immunology*, vol. 11, 2020.

Note:

- Presence of mucosal HPV at the cervix is critical for vaccine- induced immunity
- cervicovaginal secretions mainly contain IgG
- HPV biomarkers (HPV DNA) from discharged mucus and debris accumulate around urethra
- first void urine contains the most human and HPV DNA compared to random/mid-stream urine
    - first void meaning first stream of urine, not first urine of the day
- CIN2+ detection using urine shows similar sensitivity compared to clinician taking smears
- challenge in generating enough antibodies to test
- Only works in women

Conclusions/action items: This article suggested that HPV antibodies and DNA can be found in urine, however this focused on analyzing urine after vaccination. I will need to look into other non-invasive methods for HPV detection.

Josephine HALL (jrhall3@wisc.edu) - Sep 26, 2021, 9:58 PM CDT

frontiers in Immunology

OPEN ACCESS

Non-invasive Assessment of Vaccine-Induced HPV Antibodies via First-Void Urine

The potential of first-void (FV) urine as a non-invasive method to monitor human papillomavirus (HPV) vaccination has been reported, mainly focusing on urine as a sample to assess HPV DNA. Besides HPV DNA, vaccine-induced HPV antibodies originating from cervicovaginal secretions were recently shown to be detectable in FV urine as well. This presents a novel opportunity for non-invasive sampling to monitor HPV antibody status in women participating in large epidemiological studies and HPV vaccine trials. The simultaneous assessment of both HPV infection and immunogenicity on a non-invasive, readily obtained sample is particularly attractive.

INTRODUCTION

Non-invasive_Assessment_of_Vaccine-Induced_HPV_Antibodies_via_First-Void_Urine.pdf(143.5 KB) - download

9/26/2021 - Secretory immunoglobulin A in saliva of women with oral and genital HPV infection

Josephine HALL (jrhall3@wisc.edu) - Sep 29, 2021, 9:12 PM CDT

Title: Secretory immunoglobulin A in saliva of women with oral and genital HPV infection

Date: 9/26/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Investigate the potential use of saliva as a non-invasive screening method for HPV

Content:

Pubmed

Searched "HPV saliva tests"

Citation:

> A. K. Gonçalves, P. Giraldo, S. Barros-Mazon, M. L. Gondo, R. L. Amaral, and C. Jacyntho, “Secretory immunoglobulin a in saliva of women with oral and genital HPV infection,” *European Journal of Obstetrics & Gynecology and Reproductive Biology*, vol. 124, no. 2, pp. 227–231, 2006.

Notes:

- Infection due to high risk HPV strains such as HPV 16 and 18
- HPV clinical and sub clinical manifestations more often found in genital area than oral area
- immunological status is major determining factor for PV progression/reccurance
- Secretory immunoglobulin A (sIgA) - may be able to prevent HPV in oral mucosa
  - Mainly found in saliva, genitourinary secretions
- Tested 70 women with genital HPV and 70 women without HPV
- All women tested for Oral HPV DNA
- Oral swabs used - PCR
- Oral HPV in 29 women (26 of which had genital HPV)
- Oral HPV, Genital HPV, and smokers had much lower levels of sIgA
- oral mucosa very similar to genital mucosa

Conclusions/action items: This article helped to confirm that a saliva test can be used to detect HPV. This saliva test found that those with oral or genital HPV have lower levels of sIgA. This could be useful as we move forward with a saliva based test.

Josephine HALL (jrhall3@wisc.edu) - Sep 26, 2021, 10:31 PM CDT

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European Journal of Obstetrics & Gynecology and Reproductive Biology 124 (2006) 227–231

OBSTETRICS & GYNECOLOGY

www.elsevier.com/locate/ejogrb

Secretory immunoglobulin A in saliva of women with oral and genital HPV infection

Ana Katherine S. Gonçalves [a,b], Paulo Giraldo [a,b,*], Silvia Barros-Mazon [a,b], Marisa Lins Gondo [a,b], Rose Luce Amaral [a,b], Cláudia Jacyntho [a,b]

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Received 29 January 2005; received in revised form 11 May 2005; accepted 30 June 2005

Abstract

Secretory IgA contributes towards the protection of mucosal surfaces against invading microorganisms.

Objectives: Quantify secretory IgA titers in the saliva of women with HPV in the oropharynx and/or in the genital area.

Subjects and methods: Seventy women with clinical genital HPV lesions and 70 women without HPV infection were tested for oral HPV DNA and the levels of total IgA in their saliva. One milliliter of saliva was collected, centrifuged and stored at −80 °C for the measurement of secretory IgA by nephelometry technique. A pool of oral/pharyngeal cells were tested for HPV identification by polymerase chain reaction.

Results: Oral HPV PCR was positive in 29 [illegible] women (26 women with genital HPV and only 3 women without genital HPV). Titers of secretory IgA were extremely lower in patients with HPV DNA in the oropharynx when compared to HPV negative women ($p < 0.0001$). Genital HPV and smoking were also associated to low levels of total sIgA in saliva ($p < 0.01$). After multivariable analysis only the presence of HPV in the oral cavity and/or in genital area, but not smoking, was related to low levels of total secretory IgA.

Conclusion: Women with low levels of total secretory IgA could be more susceptible to having their oral mucosa colonized by HPV.

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Keywords: Human Papillomavirus; Sexually transmitted disease; Secretory IgA; Mucosal immunity; Vaginal and oral infection

1. Introduction

Human Papillomavirus (HPV) is today one of the most prevalent sexually transmitted viruses worldwide [1]. Its specificity by the skin and mucous epithelium lead to condylomas, papillomas and malignant neoplasias [1–3]. Persistent HPV infection is a necessary factor but not sufficient itself to explain the development of cervical cancer. For the majority of the adult population, it is asymptomatic and transient, presenting no clinical or sub clinical manifestations.

Despite the high prevalence of HPV infection only a small percentage of individuals develop malignant neoplasias. The presence of the virus in the tissue is not enough to explain the transformation of an infectious process into a neoplasia, even when the infection is due to high risk types such as HPV 16 or HPV 18 [3,4]. In addition to the virus, other factors are necessary to transform the infection into a pre-neoplastic lesion. It is interesting to observe that the clinical and sub clinical manifestations of HPV occur much more frequently in the genital area than in the oropharynx. In 1996, Giraldo et al. noted by means of cytological smears of the oral cavity, that there was strong cytological evidence of HPV infection in 6% of patients with genital HPV [5].

Terai et al. [6] using PCR, observed a high prevalence ([illegible]) of HPV in the normal oral cavities of adults from both sexes.

An individual's immunological status seems to be the main determining factor for the progression and recurrence of HPV. Secretory immunoglobulin A (sIgA), known to be important in the control of various diseases on mucosal surfaces, may have a role in preventing the development of HPV infection in the oral mucosa. This immunoglobulin is

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doi:10.1016/j.ejogrb.2005.06.028

Secretory_immunoglobulin_A_in_saliva_of_womenwith_oral_and_genital_HPV_infection.pdf(95.2 KB) - download

9/29/2021 - Methylation analysis in urine fractions for optimal CIN3 and cervical cancer detection

Josephine HALL (jrhall3@wisc.edu) - Oct 04, 2021, 4:21 PM CDT

Title: Methylation analysis in urine fractions for optimal CIN3 and cervical cancer detection

Date: 9/29/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Understand potential markers for HPV that can be found in urine

Content:

Database: pubmed

searched "HPV markers in urine"

Citation:

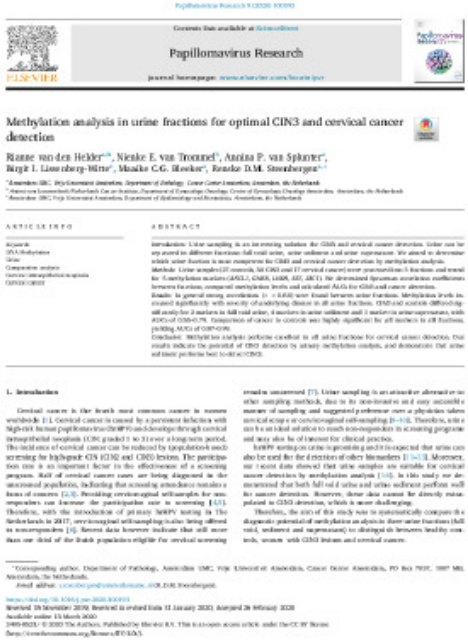
> R. van den Helder, N. E. van Trommel, A. P. van Splunter, B. I. Lissenberg-Witte, M. C. G. Bleeker, and R. D. M. Steenbergen, “Methylation analysis in urine fractions for optimal CIN3 and cervical cancer detection,” *Papillomavirus Research*, vol. 9, p. 100193, 2020.

Notes:

- full void and urine sediment can perform well in detecting cancer
- healthy controls, women with CIN 3, and women with cervical cancer
- collected in tubes containing 0.6M Ethylenedisminetraacetic acid (EDTA) - maintains DNA quality during transport
- urine centrifuged
- DNA isolated from urine
- Methylation levels of all markers increased with increasing disease severity - higher levels in cervical cancer
- urine sediment preferred

Conclusions/action items: This article was helpful in understanding how different sections of urine can be used for testing. I do not think that methylation will be an option for our design, but it is something to look into further.

Josephine HALL (jrhall3@wisc.edu) - Sep 29, 2021, 9:29 PM CDT



Papillomavirus Research

Methylation analysis in urine fractions for optimal CIN3 and cervical cancer detection

Rianne van den Helder, Nienke E. van Trommel, Annina P. van Splunter, Birgit I. Lissenberg-Witte, Maaike C.G. Bleeker, Renske D.M. Steenbergen

ARTICLE INFO

ABSTRACT

1. Introduction

Methylation_analysis_in_urine_fractions_for_optimal_CIN3_and_cervical_cancer_detection.pdf(863.7 KB) - download

10/3/2021 - Urine HPV in the Context of Genital and Cervical Cancer Screening-An Update of Current Literature

Josephine HALL (jrhall3@wisc.edu) - Oct 04, 2021, 3:58 PM CDT

Title: Urine HPV in the Context of Genital and Cervical Cancer Screening-An Update of Current Literature

Date: 10/3/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Understand current methods for HPV testing in urine

Content:

Pubmed

Searched "HPV markers in Urine"

Citation:

> A. Daponte, G. Michail, A.-I. Daponte, N. Daponte, and G. Valasoulis, “Urine HPV in the context of genital and cervical cancer screening—an update of current literature,” *Cancers*, vol. 13, no. 7, p. 1640, 2021.

Notes:

- 4 main points -
  - use of first void urine and purpose-designed collection devices
  - preservation medium to avoid human/HPV DNA degradation
  - using PCR based assays
  - processing sufficient volume of whole urine
- Molecular screening modalities are highly sensitive and are better protection from cervical cancer than cytology
- Self-sampling techniques are highly accepted
- Persistent high risk HPV(hrHPV) leading cause of over 90% cervical and anal cancers
- biomarkers in urine are reliable and noninvasive
  - used PCR
  - tested first void urine
- Urine testing on patient obtained sample was less sensitive than clinician - attributed this to signal amplification issues
- Other study found on vaginal self sampling that hrHPV assays based on PCR as sensitive done at home or by clinician to detect CIN 2+ and CIN 3+
- mailing out samples had high turn out
- take home tests with low reproducibility
- HPV16/18-E6 oncoprotein was detected in 30.6% cervical samples, 20.3% self collected vaginal samples, 21% urine samples
  - E6 oncoprotein detection can be used to detect invasive legions in urine
- Urine methylation also promising
- E6/E7mRNA in urine - self sampling at 44.8%
- Urine samples show good concordance in hrHPV detection compared with vaginal and cervical samples
- Women found urine easiest to collect and more confident that they collected the sample correctly
- monitoring of HPV antibodies - HPV^/11/16/18 antibodies can be found in first void urine

Conclusions/action items: This article helped to solidify our choice in selecting urine as the sampling medium for our device. It also suggested several potential markers that we can look for when designing the device. I will need to look further into the E6 oncoprotein and HPV antibodies.

Josephine HALL (jrhall3@wisc.edu) - Oct 03, 2021, 2:01 PM CDT

cancers

Review

Urine HPV in the Context of Genital and Cervical Cancer Screening—An Update of Current Literature

Alexandros Daponte [1,*], George Michail [2], Athina-Ioanna Daponte [3], Nikoletta Daponte [4] and George Valasoulis [1,3]

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[2] Department of Obstetrics & Gynaecology, University Hospital of Patras, 26504 Patras, Greece; georgemichail@upatras.gr
[3] Hellenic National Public Health Organization—KEDC, Marousi, 15123 Athens, Greece
* Correspondence: dapontemd@med.uth.gr; Tel.: +30-6976-568-089

Simple Summary: Despite the substantial scientific evolution in cervical cancer prevention and related infrastructures, a problem of women still resists the opportunity to detect their precancerous lesions at a curable stage by not participating in existing screening programs. Implementing sensitive screening modalities combined with easy sampling methods with minimal pain or discomfort such as self-sampling of vaginal and urine samples is increasingly applied. Self-sampling HPV modalities aimed to address this inequity, besides facilitating HPV genotyping as well as the measurement of related biomarkers in HPV-caused lesions and genital cancer. The low costs inflicted, the non-invasive nature, and the favorable acceptability profile of urine HPV detection give the potential to become a most promising tool that could expand the possibilities in changing genital and cervical cancer prevention strategies as well as in the surveillance and management of genital lesions cases.

Abstract: Within the previous decades, following the widespread implementation of HPV-related biomarkers and computerization in liquid-based cytology, screening for lower genital tract malignancies has been optimized in several parts of the world. Many organized anogenital cancer prevention systems have reached a point at which efficacy to secure a number of population coverage and less of available infrastructures. Meanwhile, self-sampling modalities in which biologic material (vaginal secretions, urine, etc.) is obtained by the individual and not the clinician and subsequently undergoes examination for HPV biomarkers enjoy appreciating acceptance. Bygone the initial skepticism that vaginal or urine HPV represents "passenger" transient infections, extensive scientific work has been conducted to optimize high-risk HPV (hrHPV) detection from this "novel" biologic material. Nowadays, several state-of-the-art meta-analyses have illustrated that self-sampling techniques involving urine self-sampling represent a feasible alternative strategy with potentially enhanced population coverage possessing excellent performance and sensitivity. Recently published scientific work focusing on urine HPV was reviewed, and after a critical appraisal, the following points should be considered in the clinical application of hrHPV urine assessments: (i) use of first-void urine (FVU) and purpose-designed collection devices; (ii) using a preservation medium to avoid harmful HPV DNA degradation during extraction and storage; (iii) using polymerase chain reaction (PCR)-based assays, ideally with genotyping capabilities; (iv) processing of a sufficient volume of whole urine; and (v) the use of an analytically sensitive HPV test/recovery of cell-free HPV DNA in addition to cell-associated DNA.

Keywords: HPV; HPV urine; cervical cancer screening; HPV DNA; genotyping; mRNA E6 & E7; HPV methylation markers

check for updates

Citation: Daponte, A.; Michail, G.; Daponte, A.-I.; Daponte, N.; Valasoulis, G. Urine HPV in the Context of Genital and Cervical Cancer Screening—An Update of Current Literature. *Cancers* **2021**, *13*, 3645. https://doi.org/10.3390/cancers13143645

Academic Editors: Raj Nath, Flavio Martin Hinds, Evangelos Paraskevaidis, Maria Kyrgiou and Ruud L.M. Bekkers

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Urine_HPV_in_the_Context_of_Genital_and_Cervical_Cancer_Screening.pdf(283.6 KB) - download

10/18/2021 - Detection of HPV E6 oncoprotein from urine via a novel immunochromatographic assay

Josephine HALL (jrhall3@wisc.edu) - Oct 19, 2021, 11:00 PM CDT

Title: Detection of HPV E6 oncoprotein from urine via a novel immunochromatographic assay

Date: 10/18/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Find information referenced in review article

Content:

Citation:

> C. M. Oliveira, L. W. Musselwhite, N. de Paula Pantano, F. L. Vazquez, J. S. Smith, J. Schweizer, M. Belmares, J. C. Possati-Resende, M. de Vieira, A. Longatto-Filho, and J. H. Fregnani, "Detection of HPV E6 oncoprotein from urine via a novel immunochromatographic assay," *PLOS ONE*, vol. 15, no. 4, 2020.

Notes:

- Used the OncoE6 Cervical test and compared to HPV DNA testing
- E6 Protein detected in 21% of urine samples
- Clinically, the E6 protein was detected in 52% of urine samples for invasive cervical cancer
- None of women in study had been vaccinated for HPV
- E6 oncoprotein from cell lyses

Conclusions/action items: Article was found and can now be effectively cited and referenced. More research needs to be done to understand when during the infection can E6 oncoprotein be detected.

Josephine HALL (jrhall3@wisc.edu) - Oct 18, 2021, 5:10 PM CDT

PLOS ONE

Detection of HPV E6 oncoprotein from urine via a novel immunochromatographic assay

Abstract

Detection_of_HPV_E6_oncoprotein_from_urine.pdf(938.5 KB) - download

10/18/2021 - First-void urine as a non-invasive liquid biopsy source to detect vaccine-induced human papilloma virus antibodies originating from cervicovaginal secretions

Josephine HALL (jrhall3@wisc.edu) - Nov 02, 2021, 9:12 PM CDT

Title: First-void urine as a non-invasive liquid biopsy source to detect vaccine-induced human papilloma virus antibodies originating from cervicovaginal secretions

Date: 10/18/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Find original article referenced in review article

Content:

Citation:

> S. Van Keer, M. Willhauck-Fleckenstein, J. Pattyn, J. Butt, W. A. A. Tjalma, X. Van Ostade, N. Hens, P. Van Damme, T. Waterboer, and A. Vorsters, “First-void urine as a non-invasive liquid biopsy source to detect vaccine-induced human papillomavirus antibodies originating from cervicovaginal secretions,” *Journal of Clinical Virology*, vol. 117, pp. 11–18, 2019.
>
> Notes:

- study of vaccinated and unvaccinated women
- also measured IgA and IgG
- tested first void urine and serum
- in both serum and urine, concentration of HPV antibodies significantly higher in vaccinated women
- only 50-70 percent of people develop humoral antibodies against HPV after natural infection
- Concentration of antibodies impacted by oral contraceptives and menstrual cycle
- at least 2 days after menstruation - take test
- GST - fusion proteins - maybe look into this more
- IgA an IgG not useful
- cervicovaginal secretions mainly contain IgG

Conclusions/action items: Article was found and can now be further reviewed. More research should be done on the GST fusion proteins mentioned

Josephine HALL (jrhall3@wisc.edu) - Oct 18, 2021, 5:22 PM CDT

Journal of Clinical Virology

First-void urine as a non-invasive liquid biopsy source to detect vaccine-induced human papillomavirus antibodies originating from cervicovaginal secretions

Severien Van Keer[a,*], Martina Willbrand-Pierkenstein[b], Jade Pattyn[a], Julia Butt[b], Wilhelmus A.A. Tjalma[c,d], Xavier Van Ostade[e], Nial Hens[a,f], Pierre Van Damme[a], Tim Waterboer[b], Alex Vorsters[a]

ARTICLE INFO

ABSTRACT

1. Background

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E-mail address: severien.vankeer@uantwerpen.be (S. Van Keer).

https://doi.org/10.1016/j.jcv.2019.05.014
Received 15 March 2019; Received in revised form 6 May 2019; Accepted 10 May 2019
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First-voidurineasanon-invasiveliquidbiopsysourcetodetectvaccine-inducedhumanpapillomavirusantibodiesoriginatingfromcervicovaginalsecretion.pdf(1.6 MB) - download

10/22/2021 - Difference in vaginal microecology, local immunity and HPV infection among childbearing-age women with different degrees of cervical lesions in Inner Mongolia

Josephine HALL (jrhall3@wisc.edu) - Oct 25, 2021, 10:25 PM CDT

Title: Difference in vaginal microecology, local immunity and HPV infection among childbearing-age women with different degrees of cervical lesions in Inner Mongolia

Date: 10/22/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Further understand and identify HPV infection biomarkers

Content:

Found on Pubmed search 'hpv antibody concentration in hpv positive women'

Citation:

> J.-J. Zheng, J.-H. Song, C.-X. Yu, F. Wang, P.-C. Wang, and J.-W. Meng, “Difference in vaginal microecology, local immunity and HPV infection among childbearing-age women with different degrees of cervical lesions in Inner Mongolia,” *BMC Women's Health*, vol. 19, no. 1, 2019.

Notes:

- As degree of cervical lesion increased, proportion of Lactobacilli decreased
  - bacterial imbalance increased, decrease in diversity and growth of normal bacteria
- IgG higher in cervical lesion group and increased as legions progressed
  - Could be used as indicator (secondary roll)
- SIgA lower in research group than control group but increased with increasing cervical cancer legions
  - Should not be used as an indicator

Conclusions/action items: This study indicated several potential secondary markers that could be used when screening for HPV. Though these markers do not test for HPV specifically, they are indicators of an imbalance which suggests an ailment. Lactobacilli and IgG could be used as secondary indicators.

BMC Women's Health

RESEARCH ARTICLE Open Access

Difference in vaginal microecology, local immunity and HPV infection among childbearing-age women with different degrees of cervical lesions in Inner Mongolia

Jing-Jing Zhong[1*], Jing-Hui Song[2*], Cong-Xiang Yu[1], Fei Wang[3], Peng-Cheng Wang[2] and Jing-Wei Meng[1]

Abstract

Background: This study aims to investigate the difference in vaginal microecology, local immunity and HPV infection among childbearing-age women with different degrees of cervical lesions.

Methods: A total of 432 patients were included in this study. Among these patients, 136 patients had LSIL, 263 patients had HSIL and 33 patients had CSCC. These patients were assigned as the research groups. In addition, 100 healthy females were enrolled and assigned as the control group.

Results: The microbiological indexes of vaginal secretions were evaluated. Furthermore, the concentrations of SIgA, IgG, IL-2 and IL-10 in vaginal lavage fluid, as well as the presence of HPV, mycoplasma and Chlamydia in cervical secretions, were detected. The results is that: (1) Differences in evaluation indexes of vaginal microecology among all research groups and the control group were statistically significant ($P < 0.0001$). As the degree of cervical lesions increased, the number of Lactobacillus decreased, and there was an increase in prevalence of bacterial imbalance, and the diversity, density and normal proportion of bacteria was reduced. Furthermore, the incidence of HPV, trichomonas, clue cell and Chlamydia infection increases. Moreover, the positive rate of H_2O_2 decreases, while the positive rates of Nia and G4DP increased. (2) Differences in the ratio of IL-2 and IL-10 in the female genital tract among all research groups and the control group were statistically significant ($P < 0.0001$).

Conclusions: As the degree of cervical lesions increases, IL-2 decreases, IL-10 increases and IL-2/IL-10 decreases, while SIgA and IgG were elevated. The reduction of dominant Lactobacillus in the vagina, impairment of H_2O_2 function, flora ratio imbalance, pathogen infections, reduction in IL-2/IL-10 ratio, and changes in SIgA and IgG levels could all be potential factors that influenced the pathogenicity of HPV infection and the occurrence and development of cervical lesions.

Keywords: Immune and HPV infection, Cervical lesions, Cervical squamous cell carcinoma, Cervical intraepithelial neoplasia, Pathogen infections

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Difference_in_vaginal_microecology_local_immunity_and_HPV_infection_among_childbearing-age_women_with_different_degrees_of_cervical_lesions_in_Inner_Mongolia.pdf(655.4 KB) - download

10/22/2021 - Correlation between HPV-negative cervical lesions and cervical microenvironment

Josephine HALL (jrhall3@wisc.edu) - Oct 25, 2021, 10:43 PM CDT

Title: Correlation between HPV-negative cervical lesions and cervical microenvironment

Date: 10/22/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Further understand and find HPV infection biomarkers

Content:

Found of Pubmed under 'hpv antibody concentration in hpv positive women'

Citation:

J.-J. Zheng, J.-R. Miao, Q. Wu, C.-X. Yu, L. Mu, and J.-H. Song, “Correlation between HPV-negative cervical lesions and cervical microenvironment,” *Taiwanese Journal of Obstetrics and Gynecology*, vol. 59, no. 6, pp. 855–861, 2020.

Notes:

- decrease un lactobacilli and dysbacteriosis as lesions progress

Conclusions/action items: This article essentially repeated the findings in the article "Difference in vaginal microecology, local immunity and HPV infection among childbearing-age women with different degrees of cervical lesions in Inner Mongolia". Lactobacilli and dysbacteriosis could also be tested for on our test strip as a disease marker, not necessarily an HPV marker

Josephine HALL (jrhall3@wisc.edu) - Oct 22, 2021, 2:20 PM CDT

Taiwanese Journal of Obstetrics & Gynecology

Original Article

Correlation between HPV-negative cervical lesions and cervical microenvironment

Jing-Jing Zheng, Jing-Rui Miao, Qiang Wu, Cong-Xiang Yu, Lin Mu, Jing-Hui Song

Correlation_between_HPV-negative_cervical_lesions_and_cervical.pdf(1.2 MB) - download

10/22/2021 - Papillomavirus E6 oncoproteins

Josephine HALL (jrhall3@wisc.edu) - Nov 02, 2021, 8:50 PM CDT

Title: Papillomavirus E6 oncoproteins

Date: 10/22/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Further understand the role and timeline E6 oncoproteins

Content:

Found on pubmed, searched, "papillomavirus e6 oncoproteins"

citation:

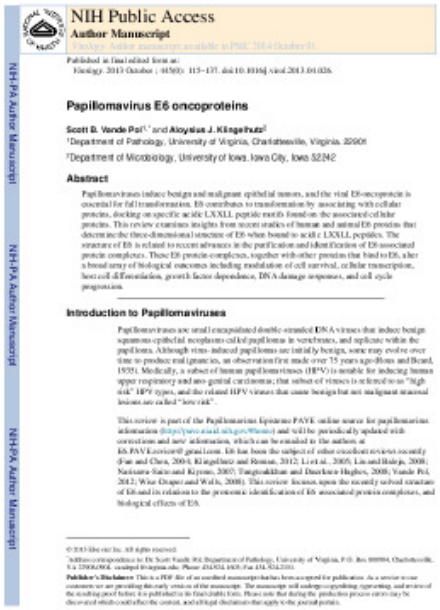
S. B. Vande Pol and A. J. Klingelhutz, “Papillomavirus E6 oncoproteins,” *Virology*, vol. 445, no. 1-2, pp. 115–137, 2013.

Notes:

- three papillomavirus early gene products (E5, E6, E7) are proteins that stimulate cell proliferation and cell survival
- continued E6 expression sustains cancer phenotype
- E6 expressed from common early promoter
- E6 may be to inhibit cell cycle arrest and apoptosis
- E6 binds alpha helical acidic LXXLL peptide expressed as part of cellular target protein
- diversity in E6-E7 region (no E6 in cows, make sure not the case in people)
- E7 from low risk viruses are weakly oncogenic directly, co-operative activity when co-expressed with high risk viruses
- Purpose of E6 to neutralize E7

Conclusions/action items: This article suggests that the presence of E6 oncoprotein found in a sample would be a cancer precursor (cancer is not far away) or an indicator that cancer is already present. This may not be the path to take for screening

Josephine HALL (jrhall3@wisc.edu) - Oct 22, 2021, 2:25 PM CDT



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Papillomavirus E6 oncoproteins

Scott D. Vande Pol[1,*] and Aloysius J. Klingelhutz[2]
[1]Department of Pathology, University of Virginia, Charlottesville, Virginia, 22908
[2]Department of Microbiology, University of Iowa, Iowa City, Iowa 52242

Abstract

Papillomaviruses induce benign and malignant epithelial tumors, and the viral E6 oncoprotein is essential for full transformation. E6 contributes to transformation by associating with cellular proteins, docking on specific acidic LXXLL peptide motifs found on the associated cellular proteins. This review examines insights from recent studies of human and animal E6 proteins that determine the three-dimensional structure of E6 when bound to acidic LXXLL peptides. The structure of E6 is related to recent advances in the purification and identification of E6-associated protein complexes. These E6 protein-complexes, together with other proteins that bind to E6, alter a broad array of biological outcomes including modulation of cell survival, cellular transcription, host cell differentiation, growth factor dependence, DNA damage responses, and cell cycle progression.

Introduction to Papillomaviruses

Papillomaviruses are small encapsidated double-stranded DNA viruses that induce benign squamous epithelial neoplasms called papillomas in vertebrates, and replicate within the papillomas. Although virus-induced papillomas are initially benign, some may evolve over time to produce real (granuloma, an observation first made over 75 years ago (Rous and Beard, 1935). Medically, a subset of human papillomaviruses (HPV) is notable for inducing human upper respiratory and ano-genital carcinomas; that subset of viruses is referred to as "high-risk" HPV types, and the related HPV viruses that cause benign but not malignant mucosal lesions are called "low-risk".

This review is part of the Papillomavirus Episteme PAVE online source for papillomavirus information (http://pave.niaid.nih.gov/#home) and will be periodically updated with corrections and new information, which can be emailed to the authors at E6.PAVE.review@gmail.com. E6 has been the subject of other excellent reviews recently (Liu and Chen, 2004; Klingelhutz and Roman, 2012; Li et al., 2005; Liu and Baleja, 2008; Sanbutra Saito and Kiyono, 2007; Tungteakkhun and Duerksen-Hughes, 2008; Vande Pol, 2012; Wise-Draper and Wells, 2008). This review focuses upon the recently solved structure of E6 and its relation to the proteomic identification of E6 associated protein complexes, and biological effects of E6.

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Papillomavirus_E6_oncoproteins.pdf(1.8 MB) - download

10/27/2021 - Human Papillomavirus E6 and E7 The Cervical Cancer Hallmarks and Targets for Therapy

Josephine HALL (jrhall3@wisc.edu) - Nov 22, 2021, 9:18 PM CST

Title: Human Papillomavirus E6 and E7: The Cervical Cancer Hallmarks and Targets for Therapy

Date: 10/27/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Understand the rolls of E6 and E7 oncoproteins

Content:

Citation:

A. Pal and R. Kundu, “Human papillomavirus E6 and E7: The cervical cancer hallmarks and targets for therapy,” *Frontiers in Microbiology*, vol. 10, 2020.

Notes:

- .initial establishment and progression of HPV induced cervical cancer depends on E6 and E7
- Manipulation of E6 and E7 are most successful form of cervical cancer therapy
- various vaccines and genome manipulation that suppress E6 and E7 have been able to decrease population of cervical cells infected with HPV
- 85% of cervical cancer deaths come from low income countries
- HPV is non-enveloped circular double stranded DNA virus
    - 300 types, 200 detrimental to humans
    - 5 groups (alpha, beta, gamma, mu, nu)
    - alpha has 65 types including 16,18,31,33,...
        - alpha responsible for 5% of cancers worldwide
    - Group 1 carcinogens are mucosal alpha which are 16,18,31,33,35,45,51,52,56,58,59
- Three vaccines available that target either 2,4, or 9 strains of HPV
- HPV 16 genome is 7.9kb long, three sections, each section divided into two polyadenylation sites
- HPV genome can get integrated with host or stay in episomal form
    - 83% of cervical cancer cases show HPV genome integrated with host
    - When integrated with host, E2 disrupted
        - E2 in charge of repressing E6 and E7
- absolute requirement of E6 and E7 for persistence of HPV caused cancer
- E7 small ~ 100 amino acids
- E6 larger ~ 150-160 amino acids
- E6 targets p53- 'guardian of the genome'
- E7 goes for pRb
- Vaccines being created to specifically target E6 and E7

Conclusions/action items: This article nicely summarized and explained how E6 and E7 work to allow for the progression of HPV caused cervical cancer. It also confirms that E6 and E7 and absolutely necessary for cancer to progress

Josephine HALL (jrhall3@wisc.edu) - Oct 27, 2021, 7:01 PM CDT

frontiers in Microbiology

Human Papillomavirus E6 and E7: The Cervical Cancer Hallmarks and Targets for Therapy

*Amrita Pal and Rita Kundu**

Cell Biology Laboratory, Department of Botany, Centre of Advanced Studies, University of Calcutta, Kolkata, India

Human papillomavirus (HPV)-induced cervical cancer is a major health issue among women from the poorly/under-developed sectors of the world. It accounts for a high-mortality rate because of its late-diagnosis and poor prognosis. Initial establishment and subsequent progression of this form of cancer are completely dependent on two major oncogenes E6 and E7, which are expressed constitutively leading to tumorigenesis. Thus, manipulation of these genes represents the most successful form of cervical cancer therapy. In the present article, information on structural, functional, and clinical dimensions of E6 and E7 activity has been reviewed. The genome organization and protein structure of E6 and E7 have been discussed followed by their mechanisms to establish the six major cancer hallmarks in cervical tissues for tumor propagation. The later section of this review article deals with the different modes of therapeutics, which functions by deregulating E6 and E7 activity. Since E6 and E7 are the biomarkers of a cervical cancer cell and are the ones driving the cancer progression, therapeutic approaches targeting E6 and E7 have been proved to be highly efficient in terms of focused removal of abnormally propagating malignant cells. Therapeutics including different forms of vaccines to advanced genome editing techniques, which suppress E6 and E7 activity, have been found to successfully bring down the population of cervical cancer cells infected with HPV. T-cell mediated immune therapy is another upcoming successful form of treatment to eradicate HPV-infected tumorigenic cells. Additionally, therapeutics using natural compounds from plants or other natural repositories, i.e., phytotherapeutic approaches have also been reviewed here, which prove their anticancer potential through E6 and E7 inhibitory effects. Thus, E6 and E7 repression through any of these methods is a significant approach toward cervical cancer therapy, described in details in this review along with an insight into the signaling pathways and molecular mechanisms of E6 and E7 action.

Keywords: human papillomavirus, oncoproteins E6 and E7, cervical cancer, cancer hallmarks, E6-targeted gene therapy, phytotherapy, immunotherapy

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INTRODUCTION

Cervical cancer is continuing to rise as a global concern with around 570,000 cases diagnosed and 311,000 deaths registered in the year 2018 (Bray et al., 2018). This form of cancer although has started to show a decline in the developed countries is still the reason behind the maximum number of cancer deaths among women in around 45 less developed countries (Torre et al., 2017).

Human_Papillomavirus_E6_and_E7_The_Cervical_Cancer_Hallmarks_and_Targets_for_Therapy.pdf(2 MB) - download

11/2/2021 - E7 oncoprotein of human papillomavirus: Structural dynamics and inhibitor screening study

Josephine HALL (jrhall3@wisc.edu) - Nov 02, 2021, 8:59 PM CDT

Title: E7 oncoprotein of human papillomavirus: Structural dynamics and inhibitor screening study

Date: 11/2/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Understand what the E& oncoprotein binds to

Content:

Citation:

> M. Aarthy, D. Kumar, R. Giri, and S. K. Singh, “E7 oncoprotein of human papillomavirus: Structural dynamics and inhibitor screening study,” *Gene*, vol. 658, pp. 159–177, 2018.

Notes:

- E6 binds to tumor suppressor p53 and inactivates it
- E7 binds to pRB tumor suppressor and inactivates it

Conclusions/action items: E7 binds to the pRB tumor suppressor, this is different than what E6 binds to. We will need to create separate testing wells for E6 and E7

Josephine HALL (jrhall3@wisc.edu) - Nov 02, 2021, 9:01 PM CDT


Gene

Research paper

E7 oncoprotein of human papillomavirus: Structural dynamics and inhibitor screening study

Murali Aarthy, Deepak Kumar, Rajanish Giri, Sanjeev Kumar Singh

E7_oncoprotein_of_human_papillomavirus__Structural_dynamics_and_inhibitor_screening_study.pdf(3.5 MB) - download

11/2/2021-Cross-Protective IgG and IgA Antibodies against Oncogenic and Non-Oncogenic HPV Genotypes

Josephine HALL (jrhall3@wisc.edu) - Nov 21, 2021, 6:40 PM CST

Title: Cross-Protective IgG and IgA Antibodies against Oncogenic and Non-Oncogenic HPV Genotypes

Date: 11/2/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Understand which HPV antibody would be most effective to test for

Content:

Citation:

A. P. Costa, P. Giraldo, R. Cobucci, M. Consolaro, R. Souza, L. B. Canário, P. Machado, R. Randall Martins, P. Vieira Baptista, J. E. Jr, and A. K. Gonçalves, “Cross-Protective IGG and IGA antibodies against oncogenic and non-oncogenic HPV genotypes,” *Asian Pacific Journal of Cancer Prevention*, vol. 21, no. 9, pp. 2799–2804, 2020.

Notes:

- Study looking at vaccinated women vs HPV positive women
- IgG antibody detected in higher quantities in vaccinated women while IgA significantly higher in those infected with HPV who are not vaccinated.
- detection done using DNA extraction

Conclusions/action items: In a population of assumed unvaccinated women, detection of IgA would be the better choice of antibody to detect. However, issues would arise in concentration levels of detection. I'm not sure how we would differentiate HPV positive concentration vs normal concentration, not a road I want to go down

Josephine HALL (jrhall3@wisc.edu) - Nov 02, 2021, 9:13 PM CDT

RESEARCH ARTICLE

Cross-Protective IgG and IgA Antibodies against Oncogenic and Non-Oncogenic HPV Genotypes

Ana Paula Costa[1], Paulo César Giraldo[2], Ricardo Noy Cobucci[3], Márcia Lopes Consolaro[4], Raquel Pantarotto Souza[4], Luanda Barbara Canário[3], Paula Renata Machado[5], Raul Randall Martins[6], Pedro Vieira Baptista[7], José Eleutério Jr[8], Ana Katherine Gonçalves[1,6]

Abstract

Introduction

Cross-Protective_IgG_and_IgA_Antibodies_against_Oncogenic_and_Non-Oncogenic_HPV_Genotypes.pdf(334 KB) - download

11/11/2021 - Small molecule activators of the p53 response

Josephine HALL (jrhall3@wisc.edu) - Nov 11, 2021, 7:39 PM CST

Title: Small molecule activators of the p53 response

Date: 11/11/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Understand what can bind to p53 aside from E6

Content:

citation:

M. J. Ladds and S. Laín, “Small molecule activators of the p53 response,” *Journal of Molecular Cell Biology*, vol. 11, no. 3, pp. 245–254, 2019.

Notes:

- p53 'guardian of the genome'
- pathway often dysregulated in cancer
    - one of most consistently mutated genes in cancer
- wild type p53 associated with positive clinical outcomes and are susceptible to chemotherapy
- p53 negatively regulated by HDM2

Conclusions/action items: This article not very helpful in understanding what else in healthy urine can bind to p53, however it did reinforce that there are often mutations to p53 with cancer present which may pose a difficulty in detection.

Josephine HALL (jrhall3@wisc.edu) - Nov 11, 2021, 7:28 PM CST

Review

Small molecule activators of the p53 response

Marcus J. G. W. Ladds[1,3,*] and Sonia Laín[1,2,*]

Small_molecule_activators_of_the_p53_response.pdf(374.5 KB) - download

11/11/2021 - Roles of pRB in the Regulation of Nucleosome and Chromatin Structures

Josephine HALL (jrhall3@wisc.edu) - Nov 22, 2021, 9:36 PM CST

Title: Roles of pRB in the Regulation of Nucleosome and Chromatin Structures

Date: 11/11/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Understand what else in healthy urine will bind/interact with pRB

Content:

Citation:

C. Uchida, “Roles of PRB in the regulation of nucleosome and chromatin structures,” *BioMed Research International*, vol. 2016, pp. 1–11, 2016.

Notes:

- LXCXE proteins can bind to the pRB protein as they have the LXCXE binding motif
- pRB involved in global epigenetic control, many things can bind and interact with it

Conclusions/action items: This article showed that there are other proteins that bind to pRb and these can be any proteins with the LXCXE. There are many things that can interact with pRb which may make it difficult to only show E7 binding instead of another protein

Josephine HALL (jrhall3@wisc.edu) - Nov 11, 2021, 7:48 PM CST

Review Article

Roles of pRB in the Regulation of Nucleosome and Chromatin Structures

Chiharu Uchida

1. Introduction

Roles_of_PRB_in_the_regulation_of_nucleosome_and_chromatin_structures.pdf(779.4 KB) - download

11/15/2021 - Urine pH: the Effects of Time and Temperature after Collection

Josephine HALL (jrhall3@wisc.edu) - Nov 15, 2021, 10:43 PM CST

Title: Urine pH: the Effects of Time and Temperature after Collection

Date: 11/15/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Establish whether pH can be used as an indicator for a 'valid' sample

Content:

Notes:

- In drug tests - urine considered invalid if pH is greater than or equal to 3 and less that 4.5, or greater than or equal to 9 and less than 11
- clinical reference interval of urine pH is 4.5-8
- urine pH will increase with increased temperature over a period of several days
- urine pH depends of time of day, diet, health, medications
    - urine more basic in the morning and more acidic at night
- urine can become contaminated with bacteria during collection
- wide range of ailments and drugs that can impact urine pH

Citation:

J. D. Cook, K. A. Strauss, Y. H. Caplan, C. P. LoDico, and D. M. Bush, “Urine ph: The effects of time and temperature after collection,” *Journal of Analytical Toxicology*, vol. 31, no. 8, pp. 486–496, 2007.

Conclusions/action items: There is such a wide range of urine pH that can be impacted by diet, medication, and health that testing the pH of a provided sample would not be a reliable indicator if the sample is effective or not.

Urine pH: the Effects of Time and Temperature after Collection*

Kathy A. Strauss

Yale H. Caplan

Urine_PH.pdf(1.7 MB) - download

11/16/2021 - Human urine - Chemical composition and fertilizer use efficiency

Josephine HALL (jrhall3@wisc.edu) - Nov 16, 2021, 10:51 PM CST

Title: Human urine - Chemical composition and fertilizer use efficiency

Date: 11/16/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Understand the chemical composition of human urine

Content:

Citation:

H. Kirchmann and S. Pettersson, “Human urine - chemical composition and fertilizer use efficiency,” *Fertilizer Research*, vol. 40, no. 2, pp. 149–154, 1995.

Notes:

- Nitrogen present as ammoniacal nitrogen, urea and uric acid in fresh urine
    - This urine had been sitting for up to 3 months
- nitrate and nitrite found as well
- in fresh urine - urea is main nitrogen compound
- Also found chlorine, potassium, sodium, phosphorous, sulfur. Small amounts of calcium and magnesium
- copper, zinc, iron, boron also found in very small amounts

Conclusions/action items: This information is useful in determining what is present in urine for selecting a control. An ion present in urine could be combined with some other substance on the test strip that could create a color change or maybe even a visual chemical reaction.

Josephine HALL (jrhall3@wisc.edu) - Nov 16, 2021, 10:42 PM CST

Human urine - Chemical composition and fertilizer use efficiency

H. Kirchmann & S. Pettersson

Abstract

Introduction

Human_urine_-_Chemical_composition_and_fertilizer_use_efficiency.pdf(420 KB) - download

11/21/2021 - Degradation of p53 Can Be Targeted by HPV E6 Sequences Distinct from Those Required for p53 Binding and Trans-Activation

Josephine HALL (jrhall3@wisc.edu) - Nov 21, 2021, 6:14 PM CST

Title: Degradation of p53 Can Be Targeted by HPV E6 Sequences Distinct from Those Required for p53 Binding and Trans-Activation

Date: 11/21/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Determine the binding affinity of E6 to p53

Content:

Citation:

T. Crook, K. H. Vousden, and J. A. Tidy, “Degradation of p53 can be targeted by HPV E6 sequences distinct from those required for p53 binding and trans-activation,” *Cell*, vol. 67, no. 3, pp. 547–556, 1991.

Notes:

- most common oncogenic genital HPV are HPV 16 and 18
- E7 shows predominant transforming and immortalizing activities in rodents, E6 cooperation necessary for immortalization of primary human epithelial cells
- only E6 and E7 region of HPV consistently retained and expressed in tumors
- E6 and E7 proteins encoded in low risk genital HPV such as HPV6 and 11
    - However, function poorly or not at all in vitro
- E6 and E7 have Cys-x-x-Cys motifs
    - 4 times in E6 and 2 times in E7
    - thought to play role in zinc binding to proteins
- E7 binds to product of retinoblastoma gene (RB)
- e6 binds to p53
- E6/E7 bind and inactivate wildtype function of proteins
- E6 binding to p53 results in p53 degradation through ubiquitin-directed system
- enhancement of p53 only in oncogenic HPV strains
- only wildtype p53 is detected in HPV positive cancers
- portion of E6 needed to bind to p53 is highly conserved
- E6 encoded by low risk HPV strains 6 and 11 also bind p53 but with lower affinity
    - HPV 6 E6 unable to direct rapid degeneration of p53 as seen in HPV 16
- N-terminal half of protein participates in rapid degradation
- No mutations in E6 binding region prevented or reduced p53 binding
    - small region of E6 between amino acids between 106 and 115 necessary for binding to p53
    - HPV 6 binding to p53 with 39% compared to HPV 16 E6
- All forms of E6 can bind p53 but benign HPV has much lower binding affinity
- correlation between ability of mutant E6 to bind p53 and the ability to enhance p53 degradation
- E6 from HPV 6 (low risk) could not enhance p53 degradation

Conclusions/action items: This article confirms that even low risk strains of HPV such as HPV 6 and 11 will produce E6 that has the ability to bind to p53. However, these non-oncogenic strains have a much lower binding affinity to p53 than oncogenic strain such as 16 and 18 do. This suggests that E6 binding will be stronger in oncogenic cases

Josephine HALL (jrhall3@wisc.edu) - Nov 21, 2021, 5:47 PM CST

Degradation of p53 Can Be Targeted by HPV E6 Sequences Distinct from Those Required for p53 Binding and Trans-Activation

Tim Crook, John A. Tidy,* and Karen H. Vousden
Ludwig Institute for Cancer Research
St. Mary's Hospital Medical School
Norfolk Place, London W2 1PG
England
*Department of Gynaecological Oncology
Samaritan Hospital for Women
Marylebone Road
London NW1 5YA
England

Summary

Introduction

T._Crook_K._H._Vousden_and_J._A._Tidy_Degradation_of_p53_can_be_targeted_by_HPV_E6_sequences_distinct_from_those_required_for_p53_binding_and_trans-activation_Cell_vol._67_no._3_pp._547_556_1991..pdf(1.7 MB) - download

11/21/2021 - High-affinity binding with specific peptides endows EuW10 a good luminescence probe for HPV E6 detection

Josephine HALL (jrhall3@wisc.edu) - Nov 29, 2021, 8:55 PM CST

Title: High-affinity binding with specific peptides endows EuW10 a good luminescence probe for HPV E6 detection

Date: 11/21/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Understand how to show a color change due to the presence of E6

Content:

Citation:

> Y. Liu, X. Yuan, W. Wang, Y. Wu, and L. Wu, “High-affinity binding with specific peptides endows EUW10A good luminescence probe for HPV E6 detection,” *New Journal of Chemistry*, vol. 42, no. 21, pp. 17339–17345, 2018.

Notes:

- Using Na9[EuW10O36]□32H2O (EuW10), concentration of 0.28 micromolar concentration of HPV16 E6 can be detected in buffer solution
- two zinc finger domains conserved across all HPV E6
- E6 expressed only in precancerous lesions and cancerous tissues
- polyoxometalates (POMs)- adjustable inorganic metal clusters
- used QKPLCPEEKQRHLDKKQR peptide sequence - E6pep
- used lab techniques for fluorescence
- E6 expressed in E. coli
- two emission peaks at 591 and 614 nm
- saturation at 15 micromolar E6
- presence of water shortens luminescence lifetime
- strong binding of EuW10 and E6 peptide
- longer lifetime if more protein

Conclusions/action items: This article shows a promising technique to view a fluorescent color change caused by E6 if lab techniques can be used. Though lab techniques cannot be used in our product, it does give a starting point for what concentration of E6 must be present for fluorescent markers to note its presence.

Josephine HALL (jrhall3@wisc.edu) - Nov 21, 2021, 6:27 PM CST

NJC

PAPER

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High-affinity binding with specific peptides endows EuW_{10} a good luminescence probe for HPV E6 detection†

Yuxue Liu, Xinxin Yuan, Weixian Wang, Yuqing Wu * and Lixin Wu *

Human papillomavirus (HPV) E6, acting as a major oncoprotein of high-risk sub-type, is known as an ideal biomarker with diagnostic potential. Due to the bottleneck lacking effective, commercially available mono-clonal antibody (mAb) for HPV16 E6, we report a new luminescence protocol for the in vitro E6 detection in the present study. By selecting an inorganic cluster, $Na_6[EuW_{10}O_{36}]\cdot 32H_2O$ (EuW_{10}) that contains rare negative charges as a fluorescence probe, a concentration as low as 0.26 pM for HPV16 E6 was detectable in buffer solution. For that, a small peptide rich in basic residues from E6 was used to construct the platform for the high-affinity interaction assay of protein to EuW_{10}. Additionally, the binding mechanism between EuW_{10} with peptide and protein was revealed in detail by time-resolved fluorescence spectrometry and ITC, while the binding processes were monitored by TEM images. Therefore, the present study not only provides a practical support for the application of polyoxometalate (POM) clusters as biological probes to HPV E6 detection, but also a significant for the early diagnosis of HPV-induced cancers.

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DOI: 10.1039/c8nj04...

Introduction

The E6 of human papillomavirus (HPV), containing about 150 amino acids, is a major oncoprotein of high-risk HPV subtypes. Its key role in developing malignant tumors has been widely recognized.[1,2] E6 targets several cell pathways to promote viral DNA replication and can form a complex with the ubiquitin ligase E6-associated protein (E6AP) and tumor suppressor protein p53, leading to ubiquitin-regulated p53 degradation. The binding with high-risk HPVs E6 can inactivate p53, prevent apoptosis, activate telomerase, disrupt cell adhesion, polarity and epithelial cell differentiation, alter transcription and G protein signaling, and reduce virus-infected cell's immune identification.[3–7]

As an ideal tar- get, the major structural feature of E6 is the presence of two atypical CX_2CX_{29}–CX_2C zinc finger domains, which are conserved in all HPV-type E6.[8,9] Just like most proteins containing multiple PDZ domains, E6 is expressed in regions where cells contact with each other. Through altering the connections between cells, it can disrupt the structure of the tissue, leading to the development of abnormal life.[4] In addition, by degrading the human anti-large tumor inhibitor (h-DLG), high-risk HPV E6 can also alter cell growth and cell contact response polarity, a sign of tumor development.[3,4] Unlike other HPV proteins that can be expressed throughout the viral life cycle, E6 is expressed only in precancerous lesions and cancerous tissues. Therefore, E6 is an ideal biomarker with diagnostic potential for cancers,[3] developing reliable tool for detection accordingly will be significant and necessary.[10] With the meanings, most useful diagnostic approaches are essentially antibodies directed against major antigens. The lack of effective, commercially available monoclonal antibody (mAb) for HPV16 E6, however, is still a challenge faced by researchers.[6–8]

Polyoxometalates (POMs) are a class of multifunctional, adjustable inorganic metal clusters.[11–14] The diversity in structures and properties endow them with wide applications in chemistry, materials science, and medicine.[15,16] The negatively charged surface of POMs leads them to easily interact with basic amino acids or positive region of proteins.[17] A series of studies on the interaction of POMs with human serum albumin (HSA) have been reported.[11,18] In addition, several POMs with unique optical properties have also been used as new candidates for biomarkers and protein detection.[19,20] For example, Eu-containing POMs have been used as attractive bio-probes for bovine HI detection.[19] Recently, we reported the interaction of cationic peptides of HPV capsid proteins with different types of Eu-containing POMs.[17,21] Through the interaction with HPV L1 peptides, POMs have been use a simple, effective and low-cost fluorescence probe to detect positively charged HPV capsid peptides[17,22] and proteins[9] in vitro, and particularly to

Y._Liu_X._Yuan_W._Wang_Y._Wu_and_L._Wu_High-affinity_binding_with_specific_peptides_endows_EUW10A_good_luminescence_probe_for_HPV_E6_detection_New_Journal_of_Chemistry_vol._42_no._21_pp._17339_17345_2018..pdf(2.2 MB) - download

11/22/2021 - Lateral Flow and Consumer Diagnostics (Pregnancy Test Methods)

Josephine HALL (jrhall3@wisc.edu) - Nov 29, 2021, 9:39 PM CST

Title: Lateral Flow and Consumer Diagnostics (Pregnancy Test Methods)

Date: 11/22/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Understand the mechanisms that allow for antibody binding in pregnancy tests

Content:

Citation:

D. Wild and S. Tiplady, “Lateral Flow and Consumer Diagnostics ,” in *The immunoassay handbook*, Amsterdam: Elsevier, 2013, pp. 533–536.

Notes:

- lateral flow immunochromatographic assays
  - two major components - wicking material that mobile labeled reagent is, membrane where other reagents are immobilized
- two main assays - sandwich assay and competitive assay
  - Sandwich for molecules larger enough for two antibodies to bind simultaneously
  - Competitive for smaller molecules only found using one antibody
- low cost, fast results (1-3 minutes)
- hCG shows up 9-10 days post conception
  - uses sandwich assay
  - one antibody immobilized on nitrocellulose test strip, second antibody labeled with colored marker while freely mobile but upstream of first antibody
  - mobile antibody has blue-colored latex particles with monoclonal antibody to alpha subunit of hCG
  - control antibodies coated in rabbit IgG also upstream
  - with addition of urine, antibodies carried to result region where other antibodies are immobilized
  - result zone has monoclonal antibody for beta subunit of hCG, this is immobilized
  - hCG reacts with anti-alphahCG antibody on latex and gets trapped by anti-betahCG antibody which causes blue line to show
  - unbound latex contacts control zone and binds with goat anti-rabbit immunoglobin antibody immobilized to membrane
  - latex bound with rabbit IgG gets trapped and control line shows up
- rapid assay use latex particles or gold sol

Conclusions/action items: This article explained how lateral flow assays work and can be used. We will want to do a lateral flow assay for our design and will likely use the same control antibodies as the pregnancy test. We will then also bind the latex dye to an antibody that would then bind to E6/E7.

Josephine HALL (jrhall3@wisc.edu) - Nov 22, 2021, 3:38 PM CST

CHAPTER 7.3

LATERAL FLOW AND CONSUMER DIAGNOSTICS

Sarah Tiplady (for enquiries, please contact fiona.hamilton@rapidpath.com)

Lateral flow immunochromatographic assays utilize porous membranes, antibodies (monoclonal and/or polyclonal), and usually a visible signal generating species to produce sensitive, disposable, and easy to use tests. This technology has been widely exploited, being utilized in a number of different applications including rapid diagnostic tests for pregnancy, fertility, drugs of abuse, and infectious disease as well as DNA detection. Similar tests are available both over-the-counter (OTC) and at point-of-care (POC). To date, a wide range of lateral flow assays have been developed for use with different sample fluids including urine, blood, plasma, serum, and saliva.

Tests based on this technology are made up of two key materials—a wicking material onto which the mobile labeled reagent is deposited and a membrane onto which the other key reagents are immobilized. Fluid flow is maintained through these materials by means of capillary forces that allow simple biochemical reactions to occur in order to generate a visible end point. One of the key features of lateral flow technology is its ability to generate a test result in a one-step procedure simply requiring the addition of the sample to the test device. There are two main lateral flow assay formats, the sandwich assay used for the detection of molecules large enough for two antibodies to bind simultaneously to the analyte and the competitive assay for detecting smaller molecules (haptens), which can only be bound by a single antibody.

Lateral flow technology lends itself well to rapid diagnostic testing because it has the benefit of ease of sampling, result interpretation, and manufacturability as well as the ability to provide results quickly (typically 1–3 min) and at a relatively low cost of goods. It is therefore not surprising that many companies such as SPD Swiss Precision Diagnostics GmbH have taken the basic principles of this technology and used it to develop rapid, reliable, and easy to use diagnostic products such as Clearblue pregnancy and fertility tests.

In 1988, Unipath Ltd, the former owners of the Clearblue brand, launched the first one-step rapid lateral flow assay, a urine-based pregnancy test simple enough for use by the untrained consumer. Prior to this, home pregnancy testing kits were akin to laboratory tests involving test tubes, multiple stages, and variable, complex chemical processes, with an analogous, hard to interpret end point. The time taken to reach this end point, at which time the test result could be read, was often as long as 2 h. Not surprisingly, few women used these home kits. The introduction of quick, easy to use, and reliable home pregnancy tests over the years has helped to build the global pregnancy and ovulation test market to a value of over $1 billion.

PREGNANCY TESTS

OTC pregnancy tests such as the Clearblue branded products detect the presence or urinary levels of human chorionic gonadotropin (hCG), which is a clinically accurate marker of pregnancy. Levels of hCG rise rapidly and predictably in the earliest days of pregnancy (Nirpotnanathy *et al.*, 2008; Johnson *et al.*, 2009), and it usually first appears in urine 9–10 days following the estimated day of conception (Wilcox *et al.*, 1999). This makes hCG an ideal urinary marker for quickly and accurately assessing whether a woman is pregnant or not.

The Clearblue pregnancy tests are based on lateral flow technology employing two monoclonal antibodies specific for hCG. One of the antibodies is immobilized in a result zone on a nitrocellulose test strip, while the second antibody is labeled with a colored marker and is located upstream of the result zone but remains freely mobile. The mobile reagent consists of blue-colored latex particles sensitized with mouse monoclonal antibody to the α-subunit of hCG. This first population of particles is used to help detect the presence of hCG in the sample. A second population of particles coated in rabbit IgG is also present; these particles are used to form a control zone, downstream of the result zone. On addition of a test sample, the labeled antibody is mobilized from the material upon which it is deposited, mixed with the sample, and carried down the test strip where it flows by capillary action through the result zone. The result zone is composed of monoclonal antibody to the β-subunit of the hCG molecule, which is immobilized in a line onto the membrane. If the urine sample contains hCG (indicative of pregnancy), the hCG reacts with the anti-hCG antibody on the latex and allows this to be trapped by the anti-hCG antibody zone on the membrane causing a blue line to appear. This is an example of an immunometric (sandwich) assay in which the intensity of the colored line at the result zone is proportional to the concentration of hCG in the urine sample. When very high levels of hCG are present and all antibody binding sites in the test have been saturated, the strength of signal reaches a plateau. When levels of hCG rise beyond this point, the sandwich assay suffers from what is known as the high-dose hook effect. At this point, the signal begins to decline because binding sites in the result zone are occupied with analyte before the analyte bound to the label has time to reach it. Again, the rate of decline is proportional to the analyte concentration in the sample (see Fig. 1).

Any unbound latex and urine continue to move along the strip by capillary action and come into contact with the control zone. This zone typically (but not in all assays) consists of a goat anti-rabbit immunoglobulin antibody that is immobilized in the membrane. Latex sensitized with rabbit IgG is trapped here, leading to the appearance of a blue line in the control zone, which appears every time the test is run, whether hCG is present or not. This internal control system demonstrates to the user that the test has been performed and has run correctly. The mechanism by which the Clearblue pregnancy test works is shown in Fig. 2.

Since its introduction, numerous manufacturers have created POC tests for detecting hCG that employ lateral

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3-s2.0-B9780080970370000361-main.pdf(472 KB) - download

11/22/2021 - Simple Telemedicine for Developing Regions: Camera Phones and Paper-Based Microfluidic Devices for Real-Time, Off-Site Diagnosis

Josephine HALL (jrhall3@wisc.edu) - Nov 29, 2021, 10:21 PM CST

Title: Simple Telemedicine for Developing Regions: Camera Phones and Paper-Based Microfluidic Devices for Real-Time, Off-Site Diagnosis

Date: 11/22/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Understand how paper can be used to detect proteins

Content:

Citation:

A. W. Martinez, S. T. Phillips, E. Carrilho, S. W. Thomas, H. Sindi, and G. M. Whitesides, “Simple telemedicine for developing regions: Camera phones and paper-based microfluidic devices for real-time, off-site diagnosis,” *Analytical Chemistry*, vol. 80, no. 10, pp. 3699–3707, 2008.

Notes:

- system should be inexpensive, requires little to no electricity, adaptable to range of conditions, simple enough for general population to understand, fast, accurate, quantitative
- paper-based diagnostic - notably in form of lateral flow immunochromatographic tests
- paper can be patterned into channels of hydrophilic paper separated by hydrophobic walls - can be used as microfluidic device for testing multiple analytes simultaneously
- urine is most informative physiological fluid that can be obtained noninvasively
- protein assay based on nonspecific binding of tetrabromophenol blue (TBPB) to proteins
    - TBPB binds to proteins through electrostatic (sulfonate) and hydrophobic (biaryl quinone methide) interactions
    - when bound phenol in TBPB deprotonates and dye shifts from yellow to blue
- used camera phones to transmit test results to lab/professional
- system has central channel that wicks sample into paper and 4 side channels that directs the sample into 4 separate test zones containing reagents for assay
- glucose assay uses diamond shaped regions to concentrate reagent
- protein assay used rectangular shape
- spotted reagents - protein used 0.2 microliters of 250mM citrate buffer, dried for 10 min , 0.2 microliters of 9mM TBPB in ethanol
- used 5 microliters of artificial urine (1 drop)
- 20 minutes for color to develop
- contaminants had little effect (dirt, sawdust, pollen)
- 30 day shelf life

Conclusions/action items: This paper showed an additional low cost paper assay that does not require lab equipment and is geared towards developing countries. This technology could be helpful in the creation of our test strip if we decide to not use antibodies.

Josephine HALL (jrhall3@wisc.edu) - Nov 22, 2021, 4:31 PM CST

Simple Telemedicine for Developing Regions: Camera Phones and Paper-Based Microfluidic Devices for Real-Time, Off-Site Diagnosis

Simple_telemedicine_for_developing_regions.pdf(817.7 KB) - download

11/29/2021 - The utility of six over-the-counter (home) pregnancy tests

Josephine HALL (jrhall3@wisc.edu) - Nov 29, 2021, 10:37 PM CST

Title: The utility of six over-the-counter (home) pregnancy tests

Date: 11/29/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Determine the smallest amount of hCG that a pregnancy test can detect

Content:

citation:

> L. A. Cole, “The utility of six over-the-counter (home) pregnancy tests,” *Clinical Chemistry and Laboratory Medicine (CCLM)*, vol. 49, no. 8, pp. 1317–1322, 2011.

Notes:

- First Response - 5.5mIU/ml
- ClearBlue - 22 mIU/mL
- 1 ng/mL = 11 mIU/mL

Conclusions/action items: hCG can be detected on the level of ng/mL, this will need to be compared to the concentration of E6/E7 to determine if we can argue a similar effectiveness

Josephine HALL (jrhall3@wisc.edu) - Nov 29, 2021, 10:33 PM CST

The utility of six over-the-counter (home) pregnancy tests

Laurence A. Cole*

Department of Obstetrics and Gynecology, USA hCG Reference Service, University of New Mexico, Albuquerque, NM, USA

Abstract

Introduction

10.1515_cclm.2011.211.pdf(233 KB) - download

12/4/2021 - Structure of the E6/E6AP/p53 complex required for HPV-mediated degradation of p53

Josephine HALL (jrhall3@wisc.edu) - Dec 04, 2021, 7:37 PM CST

Title: Structure of the E6/E6AP/p53 complex required for HPV-mediated degradation of p53

Date: 12/4/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Understand the binding of E6 to p53

Content:

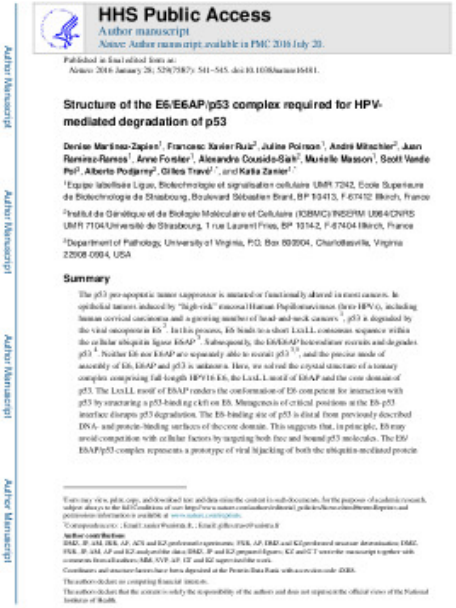
Citation:

D. Martinez-Zapien, F. X. Ruiz, J. Poirson, A. Mitschler, J. Ramirez, A. Forster, A. Cousido-Siah, M. Masson, S. V. Pol, A. Podjarny, G. Travé, and K. Zanier, "Structure of the E6/E6AP/p53 complex required for HPV-mediated degradation of p53," *Nature*, vol. 529, no. 7587, pp. 541–545, 2016.

- Notes: E6 cannot bind to p53 without first binding with E6AP
- After E6 binds to E6AP, a p53-binding cleft on E6 is created

Conclusions/action items: When creating our assay, we will attach the latex dye to E6AP antibody so that once this interacts with E6, the binding site for p53 will be ready. The p53 antibody will be immobilized on the test strip.

Josephine HALL (jrhall3@wisc.edu) - Dec 04, 2021, 7:32 PM CST



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Author manuscript

Nature. Author manuscript; available in PMC 2016 July 20.

Published in final edited form as:

Nature. 2016 January 28; 529(7587): 541–545. doi:10.1038/nature16481.

Structure of the E6/E6AP/p53 complex required for HPV-mediated degradation of p53

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[3]Department of Pathology, University of Virginia, P.O. Box 800904, Charlottesville, Virginia 22908-0904, USA

Summary

The p53 pro-apoptotic tumor suppressor is mutated or functionally altered in most cancers. In epithelial tumors induced by "high-risk" mucosal Human Papillomaviruses (hrHPVs), including human cervical carcinoma and a growing number of head-and-neck cancers[1], p53 is degraded by the viral oncoprotein E6[2]. In this process, E6 binds to a short LxxLL consensus sequence within the cellular ubiquitin ligase E6AP[3]. Subsequently, the E6/E6AP heterodimer recruits and degrades p53[4]. Neither E6 nor E6AP are separately able to recruit p53[5,6], and the precise mode of assembly of E6, E6AP and p53 is unknown. Here, we solved the crystal structure of a ternary complex comprising full-length HPV16 E6, the LxxLL motif of E6AP and the core domain of p53. The LxxLL motif of E6AP renders the conformation of E6 competent for interaction with p53 by structuring a p53-binding cleft on E6. Mutagenesis of critical positions at the E6-p53 interface disrupts p53 degradation. The E6-binding site of p53 is distal from previously described DNA- and protein-binding surfaces of the core domain. This suggests that, in principle, E6 may avoid competition with cellular factors by targeting both free and bound p53 molecules. The E6/E6AP/p53 complex represents a prototype of viral hijacking of both the ubiquitin-mediated proteo

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12/4/2021 - The Role of HPV E6 and E7 Oncoproteins in HPV-associated Cervical Carcinogenesis

Josephine HALL (jrhall3@wisc.edu) - Dec 04, 2021, 7:59 PM CST

Title: The Role of HPV E6 and E7 Oncoproteins in HPV-associated Cervical Carcinogenesis

Date: 12/4/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Find additional antibody that E7 will bind with

Content:

Citation:

E.-K. Yim and J.-S. Park, “The role of HPV E6 and E7 oncoproteins in HPV-associated cervical carcinogenesis,” *Cancer Research and Treatment*, vol. 37, no. 6, p. 319, 2005.

Notes:

- E7 does not need to bind with an additional substance before it can bind with pRb
- E7 can bind with many other substances based on chart

Conclusions/action items: When testing for E7, we will attach the latex dye to a second antibody that E7 binds to based of the E7 binding chart. We will then immobilize pRB antibody at the bottom of the test strip.

Josephine HALL (jrhall3@wisc.edu) - Dec 04, 2021, 7:56 PM CST

Cancer Res Treat. 2005;37(6):319-324 Review Article

The Role of HPV E6 and E7 Oncoproteins in HPV-associated Cervical Carcinogenesis

Eun-Kyoung Yim, Ph.D. and Jong-Sup Park, M.D., Ph.D.

Department of Obstetrics and Gynecology, The Catholic University of Korea College of Medicine, Seoul, Korea

Cervical cancer is one of the leading world causers of cancer morbidity and mortality in woman, with more than 80% related to a human papillomavirus (HPV) infection origin. Infection with specific subtypes of HPV has been strongly implicated in cervical carcinogenesis. The identification and functional verification of host proteins associated with HPV E6 and E7 oncoproteins may provide useful information in understanding cervical carcinogenesis and the development of cervical cancer-specific markers. The advent of functional genomics and proteomics has provided hope of discovering novel biological markers for use in the screening, early diagnosis, prognostication and prediction of response to therapy. Herein, we review the studies where the profiles of host proteins associated with HPV E6 and E7 oncoproteins in cervical cancer were generated. (Cancer Res Treat. 2005;37:319-324)

Key Words: Cervix neoplasms, HPV, E6, E7, Genomics, Proteomics

INTRODUCTION

319

crt-37-319.pdf(1.1 MB) - download

Binding partners	Cellular function of the binding partner
pRb	Regulation of cell-cycle control wia complex formation of E2F-transcription factors
pRb-pocket proteins	Regulation of cell-cycle control
Cyclin A, E	Kinase activity
$p21^{Cip1}$	Cyclin-dependent kinase inhibitor
$p27^{Kip1}$	Cyclin-dependent kinase inhibitor
α-glucosidase	Glycolytic control enzyme
M2 pyruvate kinase	Modulation of the activity of glycolytic enzyme 2
AP-1	Transcription factors
p48	IFN regulatioryprotein; key messenger protein
IRF-1	Regulates expression of IFN-β
Mpp2	Forkhead transcription factor
TBP	TATA box-binding protein; Initiator of transcription
TAF110	Initiator of transcription
Mi2	Histone deacetylase
S4 subunit	S4 subunit of the 26S proteasome
hTid-1	Human homolog of the *Drosophila* tumor suppressor protein Tid56
IGFBP-3	Insulin-like growth factor binding protein
Histone H1 kinase	Kinase activity

E7_binding_partners.JPG(59.9 KB) - download

9/21/2021 - Pap smear accuracy for the diagnosis of cervical precancerous lesions

Cora Williams - Oct 13, 2021, 11:18 PM CDT

Title: Pap smear accuracy for the diagnosis of cervical precancerous lesions

Date: 9/21/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Understand the pap smear as a current method for cervical cancer detection

Content:

Database: PubMed

Search "pap smear"

Citation:

E. Nkwabong, I. Laure Bessi Badjan, and Z. Sando, “Pap smear accuracy for the diagnosis of cervical precancerous lesions,” *Tropical Doctor*, vol. 49, no. 1, pp. 34–39, 2018.

Notes:

- 90% of cervical cancer deaths occur in the developing world
    - 3rd leading cause of death of women in developing countries
- CIN (cervical interaepithelial neoplasia) - 10-15 years to evolve into cancer
- Cervical dysplasia can be diagnosed through pap smear
- Pap smear is collecting superficial cells from the transformation zone and are then examined by cytopathologist
- Pap smear sensitivity is less than 70% in many studies
- study evaluating pap accuracy in diagnosing cervical precancerous lesions
- Pap and biopsy performed in all participants of study with abnormal results and macroscopic cervical changes
    - women wit found cancer at biopsy were excluded as they were looking for pre-cancerous legions
    - women who refused biopsy were also excluded
- min sample size of 44 women, ask about age, age of first sexual intercourse, age at first delivery, number of pregnancies, number of abortions and deliveries, number of sexual partners, tobacco consumption
- 75 biopsies - 54 showed cervical dysplasia
- Pap smear detected 34 abnormal cases

Conclusions/action items: This article was informative about the Pap smear procedure and its effectiveness at finding precancerous legions. However, it was somewhat difficult to interpret the data. I will be looking for further research about HPV and cervical cancer screening.

Josephine HALL (jrhall3@wisc.edu) - Sep 21, 2021, 8:56 PM CDT

14 Tropical Doctor 49(1)

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Article

Pap smear accuracy for the diagnosis of cervical precancerous lesions

Elie Nkwabong[1], Ingrid Laure Bessi Badjan[1] and Zacharie Sando[1]

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Email: elinkwabong@yahoo.fr

Pap_smear_accuracy_for_the_diagnosis_of_cervical_precancerous_lesions.pdf(132.6 KB) - download

10/4/2021 - Sampling Antibody idea

Josephine HALL (jrhall3@wisc.edu) - Oct 04, 2021, 4:02 PM CDT

Title: Sampling Antibody Idea

Date: 10/4/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Document first sampling idea

Content:

- Team could look into the HPV antibodies
  - It is known that they are found in the urine of vaccinated women at a higher rate
  - What rate are they found in women who are unvaccinated with HPV? - this could be used as a part of the test
  - Could have certain level of antibodies = certain color change or something of this nature (would need to look further into this)

Conclusions/action items: I need to do further research on the HPV antibody concentration in vaccinated and unvaccinated women with and without HPV

10/4/2021 - Collection Method Ideas

Josephine HALL (jrhall3@wisc.edu) - Oct 04, 2021, 4:08 PM CDT

Title: Collection Method Ideas

Date: 10/4/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Document collection method ideas

Content:

- **Could create a 3D printed box with a test strip or sample of reagent inside - user would urinate inside box and close the top once finished**
- **should most likely use opaque or solid colored material for testing apparatus**
- **Must be able to contain reagent during urination**
- **cheap materials - non biodegradable**
- **maybe create a shape like that of a pregnancy test**

Conclusions/action items: I will need to do more research on low cost materials that will contain the sample and not have any material property changes due to the temperature of the urine

11/2/2021-Testing apparatus design update

Josephine HALL (jrhall3@wisc.edu) - Nov 02, 2021, 9:33 PM CDT

Title: Testing apparatus design update

Date: 11/2/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Document new testing apparatus requirements

Content:

Notes:

- Testing apparatus needs to have three test strips/ slots
    - One of E6, one for E7, and one for control
- If we want to also test for HPV antibody (probably IgA)
    - would need to add another test slot

Conclusions/action items: The testing apparatus needs to be updated to have three test strips

11/16/2021 - Control line

Josephine HALL (jrhall3@wisc.edu) - Nov 16, 2021, 10:34 PM CST

Title: Control line

Date: 11/16/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: To document ideas for urine control line

Content:

Notes:

- Pregnancy tests use antibodies to detect and bind to hormones to show lines on pregnancy test
- We would need to find something found in all urine that could be detected (could use antibodies somehow)

Conclusions/action items: More research needs to be done on the chemical composition of urine

11/22/2021 - E6 and E7 antibodies Idea

Josephine HALL (jrhall3@wisc.edu) - Nov 22, 2021, 4:40 PM CST

Title: E6 and E7 Antibodies idea

Date: 11/22/2021

Content by: Josephine Hall

Present: Josephine Hall

Goals: Document the E6 and E7 Antibodies Idea

Content:

- **Instead of E6 and E7 binding to peptides, we would mimic the design on a pregnancy test and use E6 and E7 antibodies**
- **We would mark antibodies with a dye**
- **Antibodies would be secured on a line on the test strip**
  - **once E6/E7 binds to desired antibodies, color change will occur**
- **Will use same dye as pregnancy test to achieve the control line on the test to ensure that the test is working**
- **We still need to figure out:**
  - **Concentration of E6/E7 needed for antibody to bind and show up**
  - **How to bind antibody to test strip**
  - **What antibody to use for control**
  - **What dye to use**

Conclusions/action items: Dr. P pointed us in a direction of instead of binding to the peptide sequence, we can use E6/E7 antibodies and mimic a similar testing set up as a pregnancy test. We still have several things to figure out

Cervical Cancer Causing HPV strains

ADRIENNE SIMPSON - Oct 07, 2021, 11:40 PM CDT

Title: HPV strains that can lead to cervical cancer

Date: 10/4/21

Content by: Adrienne Simpson

Present: Adrienne Simpson

Goals: Identify potentially cancerous HPV strains to test for after sample collection

Content:

HPV-16 (high risk type)

HPV-18 (high risk type)

HPV-31

HPV-33

HPV-35

HPV-45

HPV-52

HPV-58

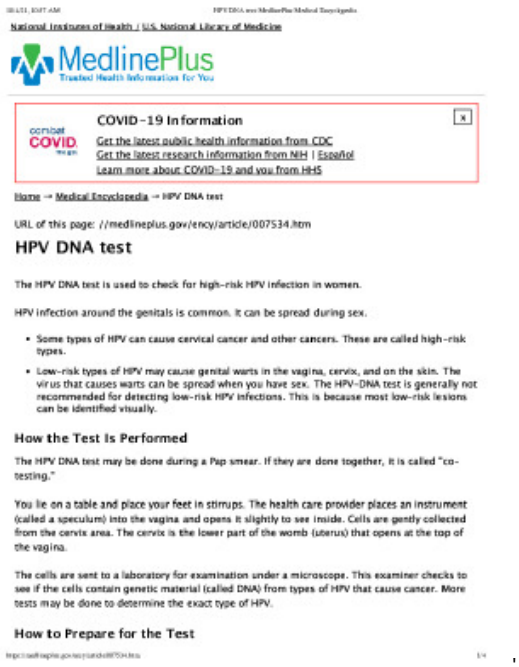
Other types of HPV strains are less common in causing cervical cancer.

Conclusions/action items:

There are 8 main HPV strains that can lead to cervical cancer in HPV positive women but only 2 of the, 16 and 18, are high risk types of HPV that are most likely to lead to cervical cancer.

L. Q. Martinez, "HPV DNA test: Medlineplus medical encyclopedia," *MedlinePlus*, 2020. [Online]. Available: https://medlineplus.gov/ency/article/007534.htm. [Accessed: 04-Oct-2021].

ADRIENNE SIMPSON - Oct 04, 2021, 10:58 AM CDT



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Home → Medical Encyclopedia → HPV DNA test

URL of this page: //medlineplus.gov/ency/article/007534.htm

HPV DNA test

The HPV DNA test is used to check for high-risk HPV infection in women.

HPV infection around the genitals is common. It can be spread during sex.

- Some types of HPV can cause cervical cancer and other cancers. These are called high-risk types.
- Low-risk types of HPV may cause genital warts in the vagina, cervix, and on the skin. The virus that causes warts can be spread when you have sex. The HPV-DNA test is generally not recommended for detecting low-risk HPV infections. This is because most low-risk lesions can be identified visually.

How the Test Is Performed

The HPV DNA test may be done during a Pap smear. If they are done together, it is called "co-testing."

You lie on a table and place your feet in stirrups. The health care provider places an instrument (called a speculum) into the vagina and opens it slightly to see inside. Cells are gently collected from the cervix area. The cervix is the lower part of the womb (uterus) that opens at the top of the vagina.

The cells are sent to a laboratory for examination under a microscope. This examiner checks to see if the cells contain genetic material (called DNA) from types of HPV that cause cancer. More tests may be done to determine the exact type of HPV.

How to Prepare for the Test

HPV_DNA_test__MedlinePlus_Medical_Encyclopedia.pdf(160.3 KB) - download

Detecting HPV Options

ADRIENNE SIMPSON - Oct 07, 2021, 11:53 PM CDT

Title: Potential Options for detecting HPV

Date: 10/4/2021

Content by: Adrienne Simpson

Present: Adrienne Simpson

Goals: To identify potential HPV detection methods

Content:

1. Vinegar/Acetic Acid Solution Test - Turns HPV infected genital areas white. It's good for detecting non-visible HPV lesions on the genitals.

Citation: "HPV infection," *Mayo Clinic*, 15-May-2021. [Online]. Available: https://www.mayoclinic.org/diseases-conditions/hpv-infection/diagnosis-treatment/drc-20351602. [Accessed: 04-Oct-2021]

2. HPV vaccine simulates HPV infection using virus-like particles (VLPs) that are formed by HPV surface components to induce antibody production in body. Maybe test for VLPs in the urine or blood to detect HPV in a person

Citation: "Human papillomavirus (HPV) vaccines," *National Cancer Institute*, 2021. [Online]. Available: https://www.cancer.gov/about-cancer/causes-prevention/risk/infectious-agents/hpv-vaccine-fact-sheet. [Accessed: 04-Oct-2021]

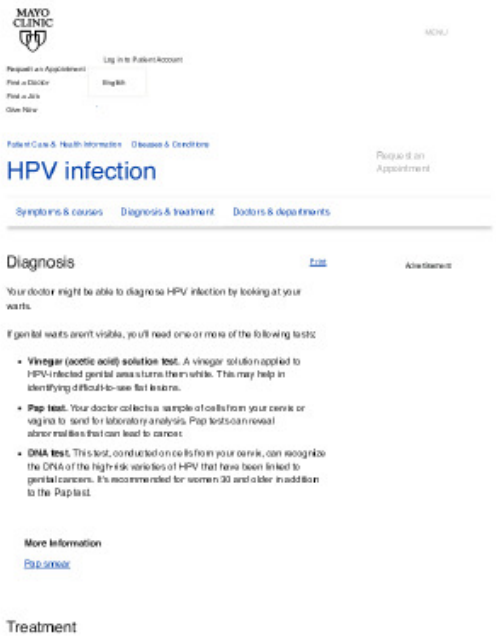
3. L1 protein capsid (found in 6 strains of HPV) can possibly be targeted. Done in vaccines.

Citation: A. Touze, S. El Mehdaoui, P. Y. Sizaret, C. Mougin, N. Muñoz, and P. Coursaget, "The L1 major capsid protein of human papillomavirus type 16 variants affects yield of virus-like particles produced in an insect cell expression system," *Journal of clinical microbiology*, Jul-1998. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC104976/. [Accessed: 04-Oct-2021].

Conclusions/action items:

Identify potential testing methods for HPV detection.

ADRIENNE SIMPSON - Oct 04, 2021, 10:59 AM CDT



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HPV infection

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Your doctor might be able to diagnose HPV infection by looking at your warts.

If genital warts aren't visible, you'll need one or more of the following tests:

- **Vinegar (acetic acid) solution test.** A vinegar solution applied to HPV-infected genital areas turns them white. This may help in identifying difficult-to-see flat lesions.
- **Pap test.** Your doctor collects a sample of cells from your cervix or vagina to send for laboratory analysis. Pap tests can reveal abnormalities that can lead to cancer.
- **DNA test.** This test, conducted on cells from your cervix, can recognize the DNA of the high-risk varieties of HPV that have been linked to genital cancers. It's recommended for women 30 and older in addition to the Pap test.

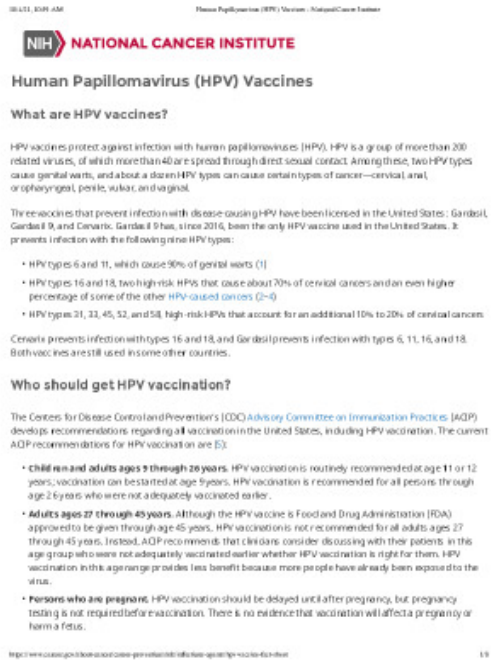
More Information

Pap smear

Treatment

HPV_infection_-_Diagnosis_and_treatment_-_Mayo_Clinic.pdf(317.8 KB) - download

ADRIENNE SIMPSON - Oct 04, 2021, 10:59 AM CDT



NIH NATIONAL CANCER INSTITUTE

Human Papillomavirus (HPV) Vaccines

What are HPV vaccines?

HPV vaccines protect against infection with human papillomaviruses (HPV). HPV is a group of more than 200 related viruses, of which more than 40 are spread through direct sexual contact. Among these, two HPV types cause genital warts, and about a dozen HPV types can cause certain types of cancer—cervical, anal, oropharyngeal, penile, vulvar, and vaginal.

Three vaccines that prevent infection with disease-causing HPV have been licensed in the United States: Gardasil, Gardasil 9, and Cervarix. Gardasil 9 has, since 2016, been the only HPV vaccine used in the United States. It prevents infection with the following nine HPV types:

- HPV types 6 and 11, which cause 90% of genital warts (1)
- HPV types 16 and 18, two high-risk HPVs that cause about 70% of cervical cancers and an even higher percentage of some of the other HPV-caused cancers (2–4)
- HPV types 31, 33, 45, 52, and 58, high-risk HPVs that account for an additional 10% to 20% of cervical cancers

Cervarix prevents infection with types 16 and 18, and Gardasil prevents infection with types 6, 11, 16, and 18. Both vaccines are still used in some other countries.

Who should get HPV vaccination?

The Centers for Disease Control and Prevention's (CDC) Advisory Committee on Immunization Practices (ACIP) develops recommendations regarding all vaccination in the United States, including HPV vaccination. The current ACIP recommendations for HPV vaccination are (5):

- **Children and adults ages 9 through 26 years.** HPV vaccination is routinely recommended at age 11 or 12 years; vaccination can be started at age 9 years. HPV vaccination is recommended for all persons through age 26 years who were not adequately vaccinated earlier.
- **Adults ages 27 through 45 years.** Although the HPV vaccine is Food and Drug Administration (FDA) approved to be given through age 45 years, HPV vaccination is not recommended for all adults ages 27 through 45 years. Instead, ACIP recommends that clinicians consider discussing with their patients in this age group who were not adequately vaccinated earlier whether HPV vaccination is right for them. HPV vaccination in this age range provides less benefit because more people have already been exposed to the virus.
- **Persons who are pregnant.** HPV vaccination should be delayed until after pregnancy, but pregnancy testing is not required before vaccination. There is no evidence that vaccination will affect a pregnancy or harm a fetus.

Human_Papillomavirus_HPV_Vaccines_-_National_Cancer_Institute.pdf(261.5 KB) - download

ADRIENNE SIMPSON - Oct 04, 2021, 5:32 PM CDT

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Journal of Clinical Microbiology

J Clin Microbiol. 1998 Jul; 36(7): 2048–2051. PMCID: PMC104975
PMID: 9650920

The L1 Major Capsid Protein of Human Papillomavirus Type 16 Variants Affects Yield of Virus-Like Particles Produced in an Insect Cell Expression System

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ABSTRACT

The L1 major capsid protein of six human papillomavirus type 16 (HPV-16) strains were expressed in insect cells by using recombinant baculoviruses. Virus-like particles (VLPs) which appeared similar to empty virions were identified by electron microscopy for all HPV strains investigated. However, the yield of VLPs produced varied in a range from 1 to 70 depending on the HPV-16 strain. The L1 proteins of these strains differed by up to 15 amino acids from the L1 protein of the prototype HPV-16 strain. Mutations in the amino acid region from residues 83 to 97 seemed to affect the level of expression of the L1 protein. These results are important when considering the development of HPV vaccines and serological tests. They indicate that strains inducing high levels of VLP production must be selected for the development of vaccines. Moreover, the L1 proteins of all strains investigated were able to bind with DNA. We also investigated the seroreactivities of VLPs derived from three different HPV-16 strains from Algeria, Senegal, and the Philippines by testing sera from women from 11 countries in immunoglobulin G–specific enzyme-linked immunosorbent assays. We observed a strong correlation between the reactivities of the three different VLP variants, independent of the geographical origin of the sera investigated. These results indicate that the three strains investigated are serologically

The_L1_Major_Capsid_Protein_of_Human_Papillomavirus_Type_16_Variants_Affects_Yield_of_Virus-Like_Particles_Produced_in_an_Insect_Cell_Expression_System.pdf(1016.3 KB) - download

Testing for HPV using Urine

ADRIENNE SIMPSON - Oct 08, 2021, 12:38 AM CDT

Title: Testing for HPV using Urine

Date: 10/8/2021

Content by: Adrienne Simpson

Present: Adrienne Simpson

Goals: To determine whether urine would be a usable alternative for HPV testing

Content:

Vaginal Discharge and Urine samples were collected from 203 women to be tested for HPV.

Vaginal Discharge results: 17.2% positive, 82.8% negative

Urine Sample results: 15.8% positive, 84.2% negative

Study showed detection of high risk HPV in urine samples was found to have a high accuracy with sensitivity of 77% and specificity of 88%

Citation: Y. S. Choi, H. Jin, and K. E. Lee, "Usefulness analysis of urine samples for early screening of human papilloma virus infection," *Journal of cancer prevention*, Dec-2019. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6951315/. [Accessed: 08-Oct-2021]

Conclusions/action items:

The study run showed that testing for cancer causing HPV strains in urine was a feasible method because cancer causing HPV was detectable in urine samples.

ADRIENNE SIMPSON - Oct 08, 2021, 12:38 AM CDT

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JCP Journal of Cancer Prevention

Usefulness Analysis of Urine Samples for Early Screening of Human Papilloma Virus Infection

Abstract

Usefulness_Analysis_of_Urine_Samples_for_Early_Screening_of_Human_Papilloma_Virus_Infection.pdf(530.5 KB) - download

E6/E7 Concentration Limit

ADRIENNE SIMPSON - Dec 01, 2021, 8:16 PM CST

Title: E6/E7 Concentration Limit

Date: 12/1/21

Content by: Adrienne

Present: Adrienne

Goals: To determine what the lowest detectable concentration of E6/E7 would be in a urine sample

Content:

Using pap smear samples, multiple tests were run to determine the optimal cut-off value for an HPV E6/E7 mRNA test when diagnosing CIN2+. The expression level of 882.53 copies/ml was found to be the optimal cut-off value for the test with a sensitivity and specificity of 79.6% and 56.9%

Y. Zhu, C. Ren, L. Yang, X. Zhang, L. Liu, and Z. Wang, “Performance of P16/KI67 immunostaining, HPV E6/E7 mrna testing, and HPV DNA assay to detect high-grade cervical dysplasia in women with ascus,” *BMC Cancer*, 27-Mar-2019. [Online]. Available: https://bmccancer.biomedcentral.com/articles/10.1186/s12885-019-5492-9. [Accessed: 02-Dec-2021].

Conclusions/action items:

The expression level of 882.53 copies/ml was found to be the optimal cut-off value when using an E6/E7 mRNA test to diagnose CIN2+

ADRIENNE SIMPSON - Dec 01, 2021, 8:10 PM CST

Performance of p16/KI67 immunostaining, HPV E6/E7 mRNA testing, and HPV DNA assay to detect high-grade cervical dysplasia in women with ASCUS

Abstract

Background

Concentration_Website.pdf(663.9 KB) - download

2014/11/03-Entry guidelines

John Puccinelli - Sep 05, 2016, 1:18 PM CDT

Use this as a guide for every entry

- Every text entry of your notebook should have the **bold titles** below.
- Every page/entry should be **named starting with the date** of the entry's first creation/activity, subsequent material from future dates can be added later.

You can create a copy of the blank template by first opening the desired folder, clicking on "New", selecting "Copy Existing Page...", and then select "2014/11/03-Template")

Title: Descriptive title (i.e. Client Meeting)

Date: 9/5/2016

Content by: The one person who wrote the content

Present: Names of those present if more than just you (not necessary for individual work)

Goals: Establish clear goals for all text entries (meetings, individual work, etc.).

Content:

Contains clear and organized notes (also includes any references used)

Conclusions/action items:

Recap only the most significant findings and/or action items resulting from the entry.

2014/11/03-Template

Cora Williams - Sep 25, 2021, 8:06 PM CDT

Title:

Date:

Content by:

Present:

Goals:

Content:

Conclusions/action items:

New Page