UNIVERSIDAD DE ALMERÍA

Doctorado en Ciencias Médicas

TESIS DOCTORAL

COMPARACIÓN DE LA EFECTIVIDAD DE LAS PRUEBAS DE AUTOMUESTREO PARA EL DIAGNÓSTICO DEL VIRUS DEL PAPILOMA HUMANO

Junio, 2022.

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COMPARISON OF THE EFFECTIVENESS OF SELF-SAMPLING TESTS FOR THE DIAGNOSIS OF HUMAN PAPILLOMA VIRUS

DOCTORANDO:
Bernardo José Vega Crespo
DIRECTORA:
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Junio, 2022.

La directora de la tesis la Profª. Dra. Gracia Castro de Luna, junto al doctorando Bernardo José Vega Crespo , garantizamos al firmar esta tesis doctoral, que el trabajo ha sido realizado por el doctorando bajo nuestra supervisión , que en la realización del trabajo se han respetado los derechos de otros autores a ser citados, cuando se han utilizado sus resultados o publicaciones, que tras la redacción, la presente memoria , que ha sido revisada por nosotros, la encontramos conforme para ser presentada y aspirar al grado de Doctor ante el Tribunal propuesto.

Y para que conste, en cumplimiento de las disposiciones vigentes, firmamos la presente en Almería a 23 de Mayo del 2022.

Directora de la Tesis **Doctorando**

Fdo. Dra. Gracia Castro de Luna Bernardo José Vega Crespo

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La presente investigación es el resultado de un esfuerzo conjunto entre la Universidad de Almería en España, Universidad de Amberes en Bélgica y la Universidad de Cuenca. La investigación se realizó con el se financiamiento del VLIR UOS (Vlaamse Interuniversitaire Raad).

El grupo de salud sexual y reproductiva de la Facultad de Ciencias Médicas de la Universidad de Cuenca, cuenta con una amplia trayectoria en el desarrollo de investigaciones e intervenciones que buscan mejorar la calidad de vida de la población en el ámbito de injerencia,

Desde el año 2010, se han desarrollado importantes aportes en la política pública en el ámbito de la salud sexual y reproductiva, así como en la detección y prevención de infecciones de transmisión sexual dentro de las cuales se encuentra el VPH. Mediante estudios poblacionales, se la logrado demostrar prevalencias altas de esta infección en poblaciones urbanas y rurales y en ciertas etnias del sur de Ecuador (Vega Crespo et al., 2020)

Pese a los esfuerzos realizados a nivel gubernamental, el acceso al cribado oportuno de cáncer de cuello uterino, sigue siendo un factor limitante, principalmente en zonas rurales del país, esta situación ha sido agravada por la pandemia de COVID 19, durante la cual los servicios de salud priorizaron la atención de esta patología (Miller et al., 2021a), limitando el acciones preventivas como el cribado del cáncer cervical.

Las técnicas de autotoma, para la detección oportuna del cáncer de cuello uterino, se presentan como una opción prometedora para eliminar las barreras organizacionales e individuales y así favorecer la adherencia al cribado.

La pregunta de investigación que guía el presente trabajo sería: ¿Cuál es la sensibilidad de las pruebas de auto toma en relación con la prueba tradicional para el diagnóstico del virus del papiloma humano?

Para responder a esta pregunta, el presente documento contiene una investigación cuantitativa, que en forma muy minuciosa expone sus objetivos, metodología, el análisis de los resultados, la discusión y las conclusiones. Por último, se ha colocado la bibliografía general en estilo APA; y, a continuación, los anexos que ayudarán al lector a complementar el entendimiento de los contenidos del estudio

RESUMEN

INTRODUCCIÓN

Las pruebas de tamizaje basada en la detección del VPH han demostrado efectividad en la prevención del cáncer; sin embargo, el examen ginecológico es considerado incómodo. Los métodos de auto muestreo incrementan la aceptabilidad del tamizaje. El objetivo de esta investigación es comparar la sensibilidad y especificidad de las pruebas la el muestreo realizado por un profesional de la salud versus la auto toma vaginal y en orina para el diagnóstico de VPH.

METODOLOGÍA

Se realizó un estudio de pruebas diagnósticas en una parroquia rural de Cuenca Ecuador, Un total de 120 mujeres participaron. Cada participante recolecto por si misma una muestra vaginal y de orina y luego tomo una prueba realizada por un profesional de salud. Esta última prueba fue considerara como el estándar de oro. Las tres muestras fueron procesadas con el mismo protocolo de amplificación e hibridación para de detección del VPH (Hybribio) siguiendo las instrucciones del fabricante.

RESULTADOS

Las características de las participantes fueron: media de edad 35 años; 40.8% casadas; 46.7% tuvieron un nivel de instrucción básica; y una media de edad de inicio de relaciones sexuales de, 17.6 años. La prevalencia de cualquier tipo de VPH fue de 15.0%, 17.5% con autotoma de orina 18.3%, con auto toma vaginal. La sensibilidad del auto muestreo alcanzó 94.4% (IC 74.2–99.9), y la especificidad 92.1% (IC 85.2–95.9). El automuestreo de orina tuvo una sensibilidad de 88.8% (IC 67.2, 96.9), y una especificidad de 94.1% (IC 67.2–96.9). El valor predictivo

negativo fue de 98.9% (IC 94.2–99.8) para el auto muestreo vaginal y de 97.6% (IC 92.6–99.4) para el auto muestreo en orina.

CONCLUSIONES

Este estudio demuestra que los métodos de auto muestreo vaginal y auto muestreo en orina tienen una sensibilidad y especificidad similar comparada con la muestra tomada por el profesional de salud para el diagnóstico del VPH, la correlación entre los tres test es satisfactoria

Palabras clave: VPH; auto muestreo vagina; auto muestreo en orina; muestreo por profesional de salud; sensibilidad y especificidad

ABSTRACT.

INTRODUCTION

HPV primary screening has shown effectiveness for cancer prevention; however, gynaecological examination is considered uncomfortable. Self-sampling methods increase the acceptance of screening. The aim of this study is to compare the sensitivity and specificity of clinician sampling versus vaginal and urine self-sampling for HPV diagnosis.

METHODS:

A diagnostic test study was conducted in a rural parish of Cuenca, Ecuador. A total of 120 women participated. Each participant self-collected urine and vaginal samples and underwent clinician sampling for HPV testing. The latter was considered as the golden standard. All three samples were processed with the same amplification and hybridization protocol for HPV detection (Hybribio) following the manufacturer's instructions.

RESULTS:

Characteristics of the participants were: median age 35 years; 40.8% married; 46.7% had a primary level of education; and median age of sexual onset, 17.6 years. The prevalence of any type of HPV with clinician sampling was 15.0%, 17.5% with urine sampling and 18.3% with vaginal self-sampling. Self-sampling sensitivity reached 94.4% (IC 74.2–99.9), and specificity 92.1% (IC 85.2–95.9). Urine sampling had a sensitivity of 88.8% (IC 67.2, 96.9), and specificity 94.1% (IC 67.2–96.9). The negative predictive value was 98.9% (IC 94.2–99.8) for vaginal self-sampling and 97.6% (IC 92.6–99.4) for urine sampling.

CONCLUSIONS:

This study shows that vaginal and urine self-sampling methods have similar sensitivity and specificity compared with clinician sampling for the diagnosis of HPV. The correlation between HPV genotypes among the three tests is satisfactory.

Keywords: HPV; self-sampling; urine sampling; clinician sampling; sensitivity and specificity

ABREVIATURAS :

CC: cáncer cervical

CAMIE: Cáncer Auto Muestreo Igualdad

DIS: Dirección Nacional de Inteligencia en Salud

DIUC: Dirección de Investigación de la Universidad de Cuenca

VPH: Virus del papiloma humano

HR: alto riesgo

PIEMB: Países de ingresos económicos medios y bajos

BR: Bajo riesgo

UC-COBIAS: Universidad de Cuenca Comité de Bioética de las áreas de la Salud)

VLIR-UOS: Vlaamse Interuniversitaire Raad Universitaire Ontwikkelingssamenwerking (Flemish Interuniversities Council University Development Co-operation)

WHO:World Health Organization

INDICE

I.INTRODUCCIÓN

1.1 Incidencia del cáncer de cervix

El cáncer de cuello (CC) continúa siendo una importante amenaza para la salud de las mujeres a nivel mundial; durante el año 2020, 604.000 nuevos casos fueron reportados a nivel mundial y más de 341.000 mujeres murieron por esta causa (Sung et al., 2021).

Aproximadamente el 90% de estos casos ocurrieron en países en vías de desarrollo. Se ha proyectado que el número de muertes se incrementará en más de un 25% en los siguientes 10 años, si no se toman medidas adecuadas para enfrentar esta enfermedad (World Health Organization. Regional Office for the Western Pacific, 2018) (World Health Organization, 2021) (Canfell et al., 2020).

El CC es un marcador de inequidades del acceso a los servicios de detección oportuna del cáncer, que divide al mundo en dos mitades. Sobre el África subsahariana las tasas de morbilidad y mortalidad por 100.000 mujeres son bajas por ejemplo Alemania 7,6; Suiza 3,4; España 5,4 en tanto que debajo de esta línea, las tasas son marcadamente más altas por ejemplo países como Zambia 65,5; Mozambique 50,2 Namibia 37,4 (Sung et al., 2021) (Arbyn et al., 2020)

En América del sur las cifras más altas de mortalidad por CC se encuentran en Bolivia, lugar donde se presenta una mortalidad de 35 x 100.000 mujeres y las más bajas en Brasil 5 x 100.000. Ecuador tiene una moralidad promedio entre los países de Sudamérica alcanzando una tasa de moralidad de 17,8/100.000 (Bruni L, Albero G, Serrano B, Mena M, Gómez D, Muñoz J, Bosch FX, de Sanjosé S., 2019). Sin embargo no se ha podido lograr una reducción en los últimos 10 años (Bruni L, Albero G, Serrano B, Mena M, Gómez D, Muñoz J, Bosch FX, de Sanjosé S., 2019) (Vega, 2012)

En Ecuador el CC es la segunda causa de muerte por cáncer ginecológico. En el año 2020, 1534 nuevos casos fueron diagnosticados a nivel nacional y 813 mujeres murieron por esta causa (Internationa Agency for Research in Cance, 2021).

En Ecuador también se pueden evidenciar inequidades, las provincias con niveles de pobreza más altos, tienen una mayor mortalidad por 100.000 habitantes: Carchi 22; Imbabura 23 así como las provincias de Chimborazo y Pastaza 10. (Vega Crespo et al., 2020)

1.2. Etiología del cáncer cervical

El cáncer de cuello uterino es una enfermedad causada principalmente por el virus del papiloma humano, sin embargo, la infección por este virus no es por si sola la causa de neoplasia pues cerca de el 80% de las mujeres sexualmente activas tienen contacto con el virus a lo largo de su vida. (Hu & Ma, 2018).

La inmunidad del huésped así como la ocongenicidad del VPH son factores que interactúan posteriores a la infección permitiendo cambios celulares que devienen en cáncer luego de un periodo de ventana que puede durar entre 5 a 10 años (Hu & Ma, 2018) (Spurgeon & Lambert, 2017).

Por ventaja entre el 60% al 90% de la infecciones por VPH son eliminadas con éxito en el lapso de 2 años posteriores a la infección gracias a la inmunidad celular a nivel cervical (Sichero et al., 2020).

Existen aproximadamente 206 subtipos de VPH; según su potencial carcinogénico se pueden clasificar según la Agencia Internacional para la Investigación en Cáncer (International Agency for Reserach on Cancer IARC) se pueden clasificar en 3 grupos:

Grupo I: incluye 13 subtipos 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, y 68 son considerados de alto riesgo (AR).

Grupo II: se encuentran 14 subtipos de posible alto riesgo (PAR) 5, 26, 53, 66, 67, 68, 70, 73, 82, 30, 34, 69, 85, y 97.

Grupo III: se encuentran principalmente 6, 11, 42, 44 considerados de bajo riesgo (BR). Éste grupo de virus se relaciona principalmente con la verrugas genitales (Hu & Ma, 2018) (Spurgeon & Lambert, 2017)

El objetivo del tamizaje, se enfoca en identificar el VPH de manera temprana para poder captar a las pacientes en ese periodo de tiempo de oro, entre la infección y el desarrollo del cáncer cervical (Hu & Ma, 2018)

1.3. Formas de prevención del cáncer de cervix.

La detección oportuna del cáncer cérvico uterino ha demostrado ser una medida costo efectiva, pues ha logrado una reducción de la mortalidad por esta causa en un 70% en los países que las han implementado para el tamizaje a grandes grupos poblacionales (McGraw, 2014a).

Mediante el desarrollo de la biología molecular, se han desarrollado diversas técnicas para un diagnostico cada vez más temprano. La detección del VPH. usado como prueba de tamizaje primario a nivel comunitario tiene una sensibilidad del 95% y una especificidad del 94% al 95%) para la detección de lesiones premalignas causadas por el VPH (Bhatla & Singhal, 2020) (Koliopoulos et al., 2017) (Nkwabong et al., 2019).comparada con la citología (Papanicolaou) que tiene una sensibilidad del 51% (Jain & Saini, 2020).

La organización mundial de la salud OMS en noviembre de 2020, lanzó la iniciativa mundial para la erradicación del cáncer de cuello uterino mediante la estrategia 90 70 90, que propone principalmente que el 90% de la población se encuentre vacunada contra el VPH; que al menos el 70% de las mujeres tengan como mínimo dos tamizajes con pruebas de alta sensibilidad, dos veces durante su vida (35 y 45 años) y que el 90% de las paciente con lesiones cervicales tengan un tratamiento adecuado (World Health Organization, 2020). Esta estrategia basa sus esfuerzos en pruebas de detección almanente sensibles mediante biología molecular, en vez de la repetición frecuente de una prueba de baja sensibilidad como es el Papanicolaou.

1.4. Nuevos enfoques en la prevención del cáncer de cervix

En América Latina, no se ha logrado una reducción del cáncer de cuello uterino en la misma proporción que los países con mayores ingresos económicos (Sichero et al., 2020) (Vega, 2012). Pese a la disponibilidad de la vacunación contra el VPH y la implementación de programas de tamizaje gratuitos, la cobertura de detección oportuna siegue siendo baja y el número de muertes por esta causa sigue siendo alto, en los países de ingresos económicos bajos (Vale et al., 2021a)

Se ha identificado varias barreras para el acceso a las pruebas de detección temprana del cáncer cervical. De acuerdo al modelo socio ecológico se han podido identificar distintos niveles en la génesis de las barreras. A nivel político se encuentran problemas en la articulación de políticas públicas e inversión para la implementación de los programas. A nivel organizacional en los servicios de salud. se han identificado barreras en el acceso tanto geográficas, como de tiempos de espera prolongados para conseguir un cita y entrega de resultados (Rodriguez et al., 2018) (Daley et al., 2011). Finalmente, a nivel individual. Las mujeres tienen una

baja adherencia al tamizaje por factores culturales, así como, la incomodidad, vergüenza y dolor que causa el examen ginecológico (Nugus et al., 2018) que se ve agravada por falta de conocimientos y precepción de riesgo de la enfermedad (Godoy et al., 2016).

El auto muestreo para el diagnóstico del VPH, en orina y en hisopado cérvico vaginal, son técnicas que han demostrado aceptabilidad y validez en diversos países (Madzima et al., n.d.) (Shin et al., 2019). Esta efectividad también ha sido demostrada en contextos rurales y con mujeres con baja adherencia al tamizaje de cáncer de cuello uterino (Yeh et al., 2019)

Existe poca evidencia sobre la efectividad del auto muestreo en términos de sensibilidad y especificidad en un contexto latinoamericano y su aceptabilidad en un contexto comunitario en zona rural,

1.5 Métodos de detección del cáncer de cervix

Desde 1970, año en que la prueba de Papanicolaou fue implementada como una herramienta de tamizaje poblacional para la detección del cáncer de cuello uterinos (CC) la mortalidad por esta causa se redujo en un 70% alrededor del mundo (Buskwofie et al., 2020). Sin embargo, la capacidad diagnóstica del Papainicolaou es relativamente baja, pues, su sensibilidad según algunos estudios varía entre el 51% al 55% y la especificidad oscila entre un 66.6% y 75% (Jain & Saini, 2020) (Nkwabong et al., 2019). Debido a estos porcentajes, el éxito de esta prueba depende de la adherencia a la repetición de la misma durante la vida de la mujer, con la finalidad de encontrar lesiones precancerosas en etapas tempranas. Para alcanzar los objetivos con ésta prueba de tamizaje, se

recomiendan intervalos de repetición para un examen de Papanicolaou que no superen los 3 años (Stumbar et al., 2019).

La detección del virus del papiloma humano (VPH), ha ganado terreno en los últimos años como método de detección primaria a nivel poblacional por su alta sensibilidad (95%) y especificidad (94 a 95%) valores superiores a los de la citología cervical o Papanicolaou (Bhatla & Singhal, 2020) (Koliopoulos et al., 2017) (Nkwabong et al., 2019). Una prueba negativa para VPH implica un riesgo incipiente para desarrollar de CC, pues, el VPH es el agente causal de esta enfermedad; su ausencia implica que no se producirán cambios celulares que desemboquen en un cáncer. Por esta razón, en las mujeres VPH negativas, se puede extender los intervalos de tamizaje a cada cinco años de acuerdo a algunos protocolos de detección (Bhatla & Singhal, 2020) (McGraw, 2014b).

La estrategia global para la prevención del cáncer de cuello uterino presentada por la Organización Mundial de la Salud (OMS), toma en cuenta la alta sensibilidad y la especificidad de los métodos diagnósticos basados en la detección mediante biología molecular y considera que la mortalidad por CC puede ser reducida efectivamente si todas las mujeres con inicio de vida sexual se realizasen al menos dos pruebas de alta sensibilidad durante su vida (34 y 45 años) (World Health Organization, 2020).

El tamizaje y la vacunación contra el VPH, son elementos, que en la actualidad han demostrado reducción en la mortalidad del cáncer de cuello uterino, principalmente en países que mantienen programas de prevención consolidados (Yang et al., 2019). Sin embargo, en aquellos países de ingresos medios y bajos (PIMB) las barreras para el tamizaje persisten (Vale et al., 2021b). El porcentaje de mujeres que no aceden al tamizaje o que no cumplen con el número de exámenes de Papanicolaou rutinarios óptimos es variable entre los países. En

Ecuador 41,6% de las mujeres en edad reproductiva nunca se han realizado una prueba de Papanicolaou (Instituto Nacional de Estadísticas y Censos, 2018).

Se han identificado varias barreras en el acceso a los servicios de tamizaje para el cáncer de cuello uterino. De acuerdo de acuerdo al modelo socio ecológico estas barreras interactúan en diferentes niveles. A nivel organizacional se incluyen: dificultades en el acceso a los servicios de salud y tiempos de espera prolongados en conseguir una cita y desde la llegada al servicio de salud hasta la atención médica. A nivel interpersonal se destaca la estigmatización de la enfermedad y falta de apoyo familiar. Finalmente a nivel individual falta de percepción de riesgo de la enfermedad y miedo a realizarse el examen (Rodriguez et al., 2018) (Sardi et al., 2019) (Balasubram, n.d.). Adicionalmente la pandemia de Covid 19 ha incrementado las disparidades en el acceso a las prestaciones de los servicios de salud (Miller et al., 2021b).

Los métodos de auto muestreo, tales como el auto muestreo cérvico vaginal y el auto muestreo en orina, han demostrado una alta aceptabilidad y sensibilidad para el diagnóstico del VPH como tamizaje primario, para la prevención del cáncer de cuello uterino (Madzima et al., 2017) (Agorastos et al., 2019). Los métodos de auto muestreo han mostrado ser menos invasivos comparados con la toma estándar realizada por un profesional de la salud, por esta razón pueden ser más atractivos para incrementar la aceptabilidad de la detección oportuna del cáncer en mujeres en mujeres no tamizadas o con un tamizaje deficiente, pues, son capaces de superar varias barreras en diferentes niveles de interacción pero principalmente mejorando la privacidad en el examen y reduciendo los tiempos de espera para la realización del examen. (Agorastos et al., 2019) (Nelson et al., 2017).

Pese a los beneficios demostrados, todavía existen dudas de la sensibilidad de los métodos de auto muestreo, estas dudas pueden desmotivar a los profesionales de la salud y a las mujeres en la decisión de utilizar este tipo de técnicas. Todavía existen limitada evidencia científica que compare la sensibilidad y especificidad del auto muestreo vaginal y en orina para la detección del VPH en la aplicación simultanea de las pruebas.

Por otro lado, pese a que los métodos de auto muestreo no son recientes, la literatura revela un amplio rango de variación de la efectividad de las pruebas en términos de sensibilidad y especificidad entre las pruebas y los autores.

En la tabla 1 se puede evidenciar que la sensibilidad de las pruebas de auto muestreo vaginal tienen una variación de 50% a 98,9% (Esber, 2018) (Kuriakose et al., 2020) y la especificidad entre 73,9% y 100% (Asciutto et al., 2018) (Kuriakose et al., 2020), De igual manera en el auto muestreo en orina la sensibilidad reportada varía entre 48,1% y 90.5% (Asciutto et al., 2018) (Cómbita et al., 2016) y la especificidad entre 74% y 82% (Asciutto et al., 2018) (Cómbita et al., 2016). Esta variación entre las investigaciones puede ser explicada por las diversas metodologías y técnicas en los estudios presentados.

Tabla 1 : Sensibilidad y especificidad de las técnicas de auto muestreo

Tipo de examen	Sensibilidad	Especificidad	Autor
Auto muestreo vaginal	83,3%	73,9%	Asciutto et al 2018
Auto muestreo vaginal	84%	93%	Arbyn et al 2018
Auto muestreo vaginal	84.6%	62.9%	Wang et al 2020
Auto muestreo vaginal	50%	98 %	Esber et al 2018
Auto muestreo vaginal	98.9%	100%	Kuriakos et al 2019
Auto muestreo en orina	48,1%	82,8%	Asciutto et al 2018
Auto muestreo en orina	90,5%	74,0%	Combita et el 2016

(Asciutto et al., 2018) (Arbyn et al., 2018) (Cómbita et al., 2016) (Arbyn et al., 2014) (Wang et al., 2020) (Esber, 2018) (Kuriakose et al., 2020)

Esta investigación es la primera que se realiza en el Ecuador, el objetivo de la misma es comparar la sensibilidad y especificidad, valores predictivos positivos y negativos, así como precisión diagnostica y correlación entre las pruebas

II JUSTIFICACIÓN

La presente investigación es el resultado de un esfuerzo conjunto entre la Universidad de Almería en España, Universidad de Amberes en Bélgica y la Universidad de Cuenca. La investigación se realizó con el se financiamiento del VLIR UOS (Vlaamse Interuniversitaire Raad).

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Desde el año 2010, se han desarrollado importantes aportes en la política pública en el ámbito de la salud sexual y reproductiva, así como en la detección y prevención de infecciones de transmisión sexual dentro de las cuales se encuentra el VPH. Mediante estudios poblacionales, se la logrado demostrar prevalencias altas de esta infección en poblaciones urbanas y rurales y en ciertas etnias del sur de Ecuador (Vega Crespo et al., 2020)

Pese a los esfuerzos realizados a nivel gubernamental, el acceso al tamizaje oportuno de cáncer de cuello uterino, sigue siendo un factor limitante, principalmente en zonas rurales del país, esta situación ha sido agravada por la pandemia de COVID 19, durante la cual los servicios de salud priorizaron la atención de esta patología (Miller et al., 2021a), limitando el acciones preventivas como el tamizaje cervical.

Las técnicas de autotoma, para la detección oportuna del cáncer de cuello uterino, se presentan como una opción prometedora para eliminar las barreras organizacionales e individuales y así favorecer la adherencia al tamizaje.

La pregunta de investigación que guía el presente trabajo sería: ¿Cuál es la sensibilidad de las pruebas de auto toma en relación con la prueba tradicional para el diagnóstico del virus del papiloma humano?

III HIPÓTESIS

La efectividad de las pruebas de auto muestreo para el diagnóstico del virus del papiloma humano, es similar a las pruebas realizadas por un profesional de la salud

IV OBJETIVOS

4.1 Objetivo general.

Comparar la sensibilidad de las pruebas de detección del virus del papiloma humano en orina y auto toma vaginal, en relación con la prueba estándar realizada por un profesional de la salud

4.2 Objetivos específicos.

1. Comparar sensibilidad, especificidad, valor predictivo positivo, valor predictivo negativo, entre la prueba de VPH en orina con la prueba estándar mediante la toma con espéculo y cepillo cervical.
2. Comparar sensibilidad, especificidad, valor predictivo positivo, valor predictivo negativo, entre la prueba de VPH mediante auto toma, con la prueba estándar mediante la toma con espéculo y cepillo cervical.
3. Evaluar los genotipos de VPH de BR

V MATERIAL Y MÉTODOS

5.1 Aprobación bioética

La presente investigación fue aprobada utilizando las recomendaciones de la declaración de Helsinki y el Consejo Internacional para Organizaciones en Ciencias Médicas (CIOMS). Todos los procedimientos que involucran seres humanos fueron aprobados por el Comité de Bioética de la Universidad de Cuenca (COBIAS) bajo el código: UC-COBIAS-2020-262) y la Dirección Nacional de Inteligencia de la Salud (DIS) del Ministerio de Salud Pública del Ecuador, bajo el código MSP-DIS-2020-0405-O. Todas las participantes fueron informadas sobre el propósito de la investigación y firmaron un consentimiento informado antes de realizar la recolección de las muestras.

5.2 Diseño del estudio

5.2.1 Población de estudio:

Participaron 120 mujeres en la evaluación de las pruebas diagnósticas, todas ellas de la parroquia rural del El Valle del cantón Cuenca, en la provincia del Azuay, Ecuador. La investigación tuvo lugar entre mayo y agosto de 2021. El reclutamiento de las participantes se realizó mediante invitaciones repartidas mediante hojas volantes en plazas públicas, domicilios y en el centro de salud de El Valle

5.2.2 Criterios de inclusión

Incluyen: haber iniciado actividad sexual; tener una edad entre 18 y menos de 70 años; no haberse sometido a procedimientos excisional o destructivos por neoplasias intraepiteliales cervicales; no haber usado medicación intra vaginal, al menos una semana antes de el examen; no haber tenido relaciones

sexuales al menos 48 horas antes del examen; no encontrase embarazada, no encontrarse con menstruación al momento de la consulta

5.3 Recolección de las muestras

Antes de recolectar las muestras, las pacientes que aceptaron participar fueron instruidas, para recolectar las muestras; un pictograma con la representación de cada técnica fue entregada a cada participante y los mismos gráficos fueron colocados en el baño donde la mujer ingresaba para recolectar la muestra de auto toma. (Anexo 1).

La primera muestra obtenida fue la de orina, la participante se recolectó la misma, de manera directa en un frasco recolector estéril, tras realizarse una asepsia de los genitales en el baño del consultorio médico. Se solicitó que cada paciente tenga la precaución de recolectar al menos 30 cc. de orina.

Tras la recolección de la orina se entregó a la paciente un dispositivo de auto toma. El dispositivo Evalyn Brush patentado por Rovers Medical Devices fue seleccionado como herramienta para el muestreo. Las instrucciones del fabricante fueron utilizadas para la recolección de la muestra. Los investigadores esperaron fuera del baño para brindar cualquier explicación adicional del procedimiento y recibir las muestras luego de haber sido recolectadas.

Finalmente, la participante fue conducida a la mesa ginecológica. Luego de la inserción del espéculo se obtuvo muestras del endocérvix y exocérvix usando el cepillo cervical de Hybirio, rotándolo 360° dos veces a nivel. El cepillo cervical, fue colocado den un recipiente de Roche Cell Collection para su

transporte. Este medio fue seleccionado debido a que contiene 20cc. de líquido preservante lo que permite que la muestra sea centrifugada y se obtenga material suficiente para el diagnóstico de VPH y el examen citológico.

Todas las muestras fueron emparejadas codificadas y trasportadas al laboratorio de biología molecular de la Universidad de Cuenca dentro de las primeras 6 horas de haber sido recolectadas. Los resultados de fueron entregados a las pacientes dentro de los primeros diez días después de su recolección, Todos los resultados en los que se pudo diagnosticar VPH de alto riesgo fueron referidas para colposcopia.

5.4 Genotipificación del virus del papiloma humano

Una vez en el laboratorio, las tres muestras de cada paciente (orina, autotoma vaginal y la muestra convencional tomada por el ginecólogo) fueron procesadas para obtener el material genético. Para la extracción del ADN de la muestra de orina, se utilizó el DNA Prep Kit (Hybribio), siguiendo las instrucciones del fabricante. Para la toma convencional, se utilizó el Cell Lysis Kit (Hybribio) siguiendo el protocolo y las indicaciones del fabricante. Finalmente, en el caso de las muestras de auto toma vaginal, los cepillos fueron lavados durante 1 minuto en el frasco que contenía el medio de recolección de Hybibrio con la finalidad de liberar las células y posteriormente realizar la extracción el ADN utilizando el mismo kit de extracción de la toma convencional. Todo el material genético obtenido fue almacenado a – 20 °C para su futuro análisis.

Para la amplificación se utilizó el kit de Hybibrio GENOARRAY para 37 genotipos de virus del papiloma humano (VPH), el cual permite la amplificación simultánea de 37 tipos diferentes de virus del papiloma humano incluyendo los genotipos de alto riesgo (AR): 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66,68, de bajo riesgo (BR): 6, 11, 42, 43, 44, CP8304 (81) y 26, 34, 40, 54, 55, 57, 61, 67, 69, 70, 71, 72, 73, 82, 83, 84 categorizados como riesgo no determinado.

La mezcla de PCR se realizó de acuerdo a las instrucciones del fabricante para obtener un volumen final de reacción de 25µl (23.25µl PCR Mix, 0.75µl de ADN Taq polimerasa 5U/µl y 1µl de ADN), Para la mezcla de las muestras de orina el volumen final fue de 26µl porque se utilizaron 2 µl de ADN.

La amplificación se realizó en un termociclador Veriti (Applied Biosystems) con la siguiente programación: Desnaturalización inicial a 95°C por 5 minuto, 40 ciclos de: desnaturalización a 95° por 20 segundos, hibridación a 55ºC por 30 segundos y elongación a 72ºC por 30 segundos, para terminar con la elongación final a 72ºC por 5 minutos.

Finalmente, todos los amplicones fueron desnaturalizados por 5 minutos a 95C° y colocados en hielo antes de continuar la hibridación. El proceso fue llevado a cabo en el dispositivo HibriMax (Hybribio) de acuerdo a las especificaciones del fabricante. Se utilizaron las membranas HPV-37 Hybrimem que contienen las sondas inmovilizadas de los genotipos del virus de papiloma humano de interés. El conjugado enzimático de estreptavidina fue añadido para unirse a los productos biotinados de la PCR. La visualización directa de los productos de la descomposición (precipitado púrpura) de el sustrato de tatrazolio nitro azul y 5-bromo-4-cloro-3´-indolifosfato fue

interpretado como positivo para el genotipo de VPH correspondiente como se indica en el diagrama esquemático de la membrana del kit. (Anexo 2)

5.5 Análisis estadístico de datos

El cuestionario completo, con los datos socio demográficos y los resultados de las pruebas de VPH fueron trascritos a una hoja de cálculo de Microsoft Excel 2016, con la finalidad de limpiar y codificar y subsecuentemente trasferidos a programa Statistical Package for the Social Sciences for Windows versión 22.0 (SPSS IBM, Armonk, NY, USA). El análisis descriptivo se realizó usando medias y desviación standard (DS) para las variables continuas y frecuencias y porcentaje para las variables categóricas. El programa Open-Source Epidemiologic Statistics for Public Health (Open Epi. Rollins School of Public Health de la Universidad de Emory, Atlanta, GA, USA) fue usado para calcular la sensibilidad, especificidad, valor predictivo positivo y negativo, razón de verosimilitud positivo y negativo, precisión diagnóstica y correlación de Kappa Cohen. La prueba estadística de Kappa fue calculada para determinar el nivel de concordancia entre los métodos. Un valor de 0 indica que no existe concordancia, un valor de 1 indica una concordancia perfecta y valores intermedio 0.00–0.20, 0.21–0.40, 0.41–0.60, 0.61–0.80 y >0.81 indican una pobre, ligera, buena y excelente correlación respectivamente.

La muestra tradicional, obtenida por el personal de salud del cuello uterino, es el método estándar para el diagnóstico del VPH, por esta razón la sensibilidad y especificidad de las pruebas diagnósticas de auto toma en orina y vaginal se calcularon usando la toma tradicional por parte del profesional como referencia. La amplitud de los intervalos de confianza de la prueba de kappa y los parámetros de efectividad se utilizaron para demostrar la precisión de cada una de las pruebas.

VI. RESULTADOS

6.1 Características de la población

Un total de 120 mujeres participaron en este estudio, todas ellas residentes en el área rural de la parroquia El Valle.

La población encuestada es mayoritariamente joven con una media de edad de 35 años con una desviación standart de 11,23; el nivel de instrucción en general es bajo el 55,1% de las participantes tiene un nivel básico de instrucción o inferior. Según su estado civil la mayoría 73,1% se encuentra en una relación estable con su pareja

Mas de la mitad de las participantes son amas de casa (57.7%); la mayoría de las participantes tiene una condición económica baja, el 70, 8% de las familias tiene ingreso inferior al sueldo básico mensual del trabajador ecuatoriano. La edad media de inicio de las relaciones sexuales fue a los 17,6 años con una DS de 2,9. El 18.3% de las participantes no se ha realizado un tamizaje. (Tabla 2).

Tabla 2: Características Socio- demográficas

Variable	N(%)
Edad	
19 a 29	42(35,5)
30 a 39	32(26,7)
40 a 49	31(25,8)
50 a 59	12(10,8)
60 a 69	2 (1,7)
Nivel de instrucción	
Ninguno	8(6,7)
Centro de alfabetización	1(0,8)
Escuela primaria	56(46,7)
Ed secundaria	43(35,8)
Ed Superior	11(9,2)
Post grado	1(0,8)
Estado Civil	
Casada	49(40,8)
Unión estable	28(23,3)
Soltera	25 (20,8)
Divorciada	11(9,2)
Separada	3(2,5)
Viuda	4(3,3)
Ocupación	
Ama de casa	69(57,5)
Empleada	27(22,5)
Agricultura	3(2,5)
Estudiante	2(1,7)
Jubilada	1(0,8)
Peluquera	1(0,8)
Comerciante	1(0,8)
Limpieza	1(0,8)
Otros	3(2,5)
Ingresos familiares mensuales (USD)	
< 100	22(18,3)
100 a 200	21(17,5)
201 a 300	19(15,8)
301 a 400	23(19,2)
401 a 500	17(14,2)
501 a 600	6(5,0)
>600	12(10,0)
Edad de inicio de las relaciones sexuales.	
9 a 14 años	12(10,0)
15 a 19 años	82(68,3)

20 a 24 años	23(19,2)
25 a 29 años	2(1,7)
30 a 34 años	1(0,8)
Tamizaje cervical previo	
Si	98(81,7)
No	22(18,3)

6.2 Positividad de virus del papiloma humano según pruebas

Del total de pruebas realizadas se encontró una positividad mayor en las pruebas de auto muestreo, para auto toma vaginal 20.8%, y para la toma de orina con 18.3% que para de la prueba tomada por el profesional de la salud con un 15% . (Tabla 3)

Tabla 3: Porcentaje de positividad del VPH según prueba realizada

Resultado de la prueba	Toma por profesional	Auto toma vaginal	Auto toma orina
Presencia de VPH	18 (15,0)	25 (20,8)	22(18,3)
No se detecta VPH	102 (85,0)	95 (79,2)	98(81,7)

La detección de poli infecciones por el VPH es más frecuentemente captada por las pruebas de auto toma vagina y en orina en comparación con la toma vaginal. El, 11,1% de las tomas por el profesional de salud y el 13,6% auto tomas vaginales y el 4.5% de auto toma en orina demostraron la coexistencia de 2 tipos de virus. Por otro, lado el 5,6% de las mujeres con la toma tradicional, presentaron la coinfección versus de más de dos virus, versus, el 4,5% con auto toma y el 9,1% con auto toma en orina (Tabla 4)

Tabla 4: Presencia de coinfecciones de genotipos de VPH según el tipo de muestreo

Tipo de prueba	Número de virus de HPV detectados					Total
	N(%)	**N(%)**	**N(%)**	**N(%)**	**N(%)**	**N(%)**
	1 virus	**2 virus**	**3 virus**	**4 virus**	**5 virus**	
Toma por profesional	15(83,3)	2(11,1)	0(0,0)	1 (5,6)	0(0,0)	18(100,0)
Auto toma vaginal	18(81,1)	3(13,6)	0(0,0)	1(4,5)	0(0,0)	25(100,0)
Auto toma orina	19(86,4)	1(4,5)	1(4,5)	0(0,0)	1(4,5)	22(100,0)

Los genotipos en general más frecuentemente identificados son: 58 51,31,52,53,16. Los detectados en la auto toma vaginal pero no en la toma tradicional son: 11,33,68,72. Por otro lado los genotipos 11,54,68 y 73, fueron detectados en la auto toma de orina pero no en la toma tradicional. (Tabla N°5).

6.3 Evaluación de las pruebas diagnósticas

La prueba de auto muestreo vaginal alcanza una sensibilidad de 94,4% (IC 74.2- 99); una especificidad de 92,1% (IC 85.2- 95.9); valor predictivo positivo de (VPP) 68,0% (IC 48.4-82.8); valor predictivo negativo (VPN) 98,9% (IC 94.28, 99.81) razón de verosimilitud positiva (RVP) 12 (IC 9,36 – 15.49); razón de verosimilitud negativa (RVN) 0,06 (IC 0,008 – 0,428). La concordancia con la prueba tomada por el profesional es de 0,74 (kappa) y la precisión diagnóstica de 92,5%

Para la prueba de orina se encontró una sensibilidad de 88.8% (IC 67.2, 96.9); especificidad 94,1% (IC 87.76, 97.28); VPP 72,2% (IC 51.85, 86.85); V¨PN 97,6% (IC 51.85- 86.85); RVP 15 (IC 10,73 – 21,27); RVN 0,11 (IC 0,04 – 0,315) la concordancia

con la prueba tomada por el profesional es de 0,76 (kappa) y la precisión diagnóstica alcanza el 93,3% (Tabla 6)

Tabla 5 Distribución de cualquier tipo de VPH de acuerdo al método de muestreo

Genotipo	11-	16*	18*	31*	33*	39*	51*	52*	53**	54-	56*	58*	66**	68*	70-	71-	72-	73*	81-	84-
Toma por el médico	-	1(4,3)	1(4,3)	3(13,0)	-	1(4,3)	3(13,0)	2 (8,7)	2(8,7)	-	1(4,3)	4(17,4)	1(4,3)	-	1(4,3)	1(4,3)	-	-	1(4,3)	1(4,3)
Muestreo vagina	1(3,2)	2(6,5)	1(6,5)	3(9,7)	1(3,2)	1(3,2)	4(12,9)	4(12,9)	2(6,5)	-	1(3,2)	5(19,4)	1(3,2)	1(3,2)	1(3,2)	1(3,2)	1(3,2)	-	-	1(3,2)
Muestro en Orina	1(3,4)	2(6,9)	1(6,9)	3(10,3)	-	1(3,4)	4(13,8)	1(3,4)	2(6,9)	2(6,9)	1(3,4))	4(13,8)	2(6,9)	1(3,4)	1(3,4)	-	-	1(3,4)	1(3,4)	1(3,4)

*VPH de alto riesgo**; VPH de mediano riesgo; - VPH de bajo riesgo

Tabla 6 Comparación de sensibilidad, especificidad, valores predictivos razón de verosimilitud correlación y precisión diagnostica de las pruebas para el diagnostico de cualquier tipo de VPH

		Toma por el médico		**Sensibilidad**	**Especificidad**	**VPP**	**VPPN**	**RVP**	**RVN**	**Kappa**	**Precisión Dx.**
	Resultado	Positivo n(%)	Negativo n(%)	% (IC 95%)	% (IC 95%)	% (IC 95%)	% (IC 95%)	n (IC)	N (IC)	n (IC)	n (IC)
Muestro Vaginal	Positivo	17(14,2)	8(6,7)	94,4 (74.2- 99.0)	92,1 (85.2- 95.9)	68,0 (48.4-82.8)	98,9 (94.28, 99.81)	12,0 (9.361 - 15.49)	0,06 (0.008 - 0.428)	0.74 (0.57 - 0.92)	92,5 (86.36,95.97)
	Negativo	1(0,8)	94(78,3)								
Muestreo en orina	Positivo	16(13,3)	6(5,0)	88,8 (67.2, 96.9)	94,1 (87.76, 97.28)	72,7 (51.85, 86.85)	97,6 (92.86, 99.44)	15,1 (10.73 - 21.27)	0,11 (0.044 - 0.315)	0.76 (0.58 - 0.93)	93,33 (87.39,96.58)
	Negativo	2(1,7)	96(80,0)								

Tabla 7: Comparación de sensibilidad, especificidad, valores predictivos razón de verosimilitud correlación y precisión diagnostica de las pruebas para el diagnóstico de los VPH de alto riesgo

		Toma por el médico		**Sensibilidad**	**Especificidad**	**VPP**	**VPPN**	**RVP**	**RVN**	**Kappa**	**Precisión Dx.**
	Resultado	Positivo n(%)	Negativo n(%)	% (IC 95%)	% (IC 95%)	% (IC 95%)	% (IC 95%)	n (IC)	N (IC)	% (IC 95%)	% (IC 95%)
Muestreo Vaginal	Positivo	12(10,0)	6(5,0)	100,0 (75.7, 100.0)	94,4 (88.4, 97,43)	66,6 (43.74, 97,43)	100,0 (96.3, 100.0)	18 (12.98, 24.95)	0.0	0,77 (0.59, 0.94)	95..00 (89.52,97.69)
	Negativo	0	102(85,5)								
Muestreo en orina	Positivo	11 (9,2)	4(3,3)	91,6 (64.6, 98.5)	96,4 (91.18, 98.6)	73,3 (48.05, 89.1)	99,0 (90.9 99.8)	25.,6 (15.4, 42.58)	0,08 (0.01,0.61)	0,79 (0.61, 096)	95,97 (90.91,98.27)
	Negativo	1(0,8)	108(86,7)								

La comparación entre las tres pruebas es más alta cuando únicamente se compara la detección de los genotipos de alto riesgo del VPH: la prueba de auto muestreo vaginal alcanza una sensibilidad de 100,0% (IC 74.2-100.0); una especificidad de 94,4% (IC 88.4- 97.4); valor predictivo positivo de (VPP) 66,6% (IC 43.7-97.4); valor predictivo negativo (VPN) 100,0% (IC 96.3, 100.0) razón de verosimilitud positiva (RVP) 18 (IC 12,9 – 24.9); razón de verosimilitud negativa (RVN) 0,0 La concordancia con la prueba tomada por el profesional es de 0,77 (kappa) y la precisión diagnóstica de 95,0%

Para la prueba de orina se encontró una sensibilidad de 91.6% (IC 64.6, 98.5); especificidad 96,4% (IC 91.1, 98.6); VPP 73,3% (IC48.0, 89.1); V¨PN 99,8% (IC 9.09 – 98.8); RVP 25.6 (IC 15,47 – 42,58); RVN 0,08 (IC 0,01 – 0,06) la concordancia con la prueba tomada por el profesional es de 0,79 (kappa) y la precisión diagnóstica alcanza el 95,9% (Tabla 7)

VII. DISCUSIÓN

El objetivo de la presente investigación fue el de comparar la sensibilidad y especificidad de las pruebas de auto muestreo con las pruebas realizadas un profesional de la salud para el diagnóstico del virus del papiloma humano

La prevalencia general del virus del papiloma humano de AR y de BR es altamente variable según la literatura consultada. En Europa el porcentaje de positividad oscila entre 2% en España y el 12% en Bélgica (De Vuyst et al., 2009). En Ecuador esta variación también está presente para Cabrera J. et al en 2015 reportaron un prevalencia del 25,6% en la provincia del Azuay de Ecuador (Cabrera V. et al., 2015); González-Andrade F. et al, en 2019 reportaron una prevalencia de 6,3% entre las mujeres mestizas de Ecuador (González Andrade et al., 2019). La presente investigación presenta una prevalencia del 15% similares a los resultados presentados por Dunne E. et al in en los Estados Unidos, donde se encuentra una prevalencia del 17% (Dunne et al., n.d.). Una posible explicación de la alta prevalencia encontrada es debida que en nuestra investigación se incluyó a un importante número de mujeres menores de 30 años (Baloch et al., 2016).

La prevalencia de cualquier tipo de VPH es ligeramente mayor en la auto toma vaginal (18,3%) y el el auto muestreo de orina (17,5%); similares resultados fueron descritos por Polman N. et.al en 2019 en Holanda, donde la positividad de VPH en el auto muestreo vaginal fue de 7,4% versus 7,2% con la toma realizada por un profesional de la salud (Polman et al., 2019). En el auto muestreo de orina y vaginal se pueden recolectarse un mayor número de células provenientes del tracto genital inferior comparadas con la toma del profesional que solo recolecta células endo y exo cervicales. Esto podría explicar el alto porcentaje de positividad en los métodos de auto muestreo

Los genotipos de VPH también presentan una amplia variación en su prevalencia en todo el mundo. En Europa los genotipos más prevalentes son: 16,18,31,33 (De Vuyst et al., 2009); y en China 52, 58, 31, 52, 39, 68 (Baloch et al., 2016). Nuestra investigación presenta resultados similares a los de González-Andrade F en Ecuador, donde se detecta que los genotipos prevalentes son 16, 18, 31, 52, 53, 56, 58. Esta variación puede ser explicada por la prevalencia epidemiológica del VPH de acuerdo al lugar de residencia de las participantes involucradas en el estudio (González Andrade et al., 2019).

Los valores de sensibilidad y especificidad de la auto toma vaginal y de orina son comparables a los hallazgos de Arbyn et al 2018 (84% y 93%) (Arbyn et al., 2018) y son inferiores a los reportados por Kuriakos et al 2019 (98,9% and 100%) (Kuriakose et al., 2020). La estandarización de la técnica puede explicar estas variaciones. En la presente investigación se realizó el análisis utilizando el mismo equipo de hibridación lo que genera que los resultados sean más homogéneos entre los test. Sin embargo, a pesar de existir diferencias entre los valores de sensibilidad las técnicas de auto muestreo han demostrado una eficiencia similar a las muestras obtenidas por un profesional de salud, (Polman et al., 2019).

Para las muestras de orina, nuestros resultados fueron similares a la sensibilidad y especificidad reportadas por Combita et al in 2016 (90,5%- 74,0%) (Cómbita et al., 2016). Sin embargo, nuestra investigación demostró que la sensibilidad fue más elevada, Esta diferencia puede también ser explicada por la técnica empleada: en nuestra investigación se utilizó un reactivo diseñado específicamente para el análisis de VPH en orina, esta situación podría explicar el incremento en la sensibilidad, no siendo considerados como falsos positivos pues la comparación se realizó con la prueba realizada por el profesional de la salud.

La prueba de Papanicolaou tiene una sensibilidad media de 51% (Jain & Saini, 2020) (Nkwabong et al., 2019). Los dos métodos de auto toma puede ser considerados más eficientes debido a que la sensibilidad es superior al 80%(Swift et al., 2020). El tamizaje mediante la detección de VPH es más efectivo y por esta razón se podría realizar menos frecuentemente que el Papanicolaou. Adicionalmente la alta especificidad y los valores predictivos negativos son relevantes para la práctica clínica , debido a que los pacientes con un resultado negativo excepcionalmente presentan una lesión cervical y por esta razón tienen menores posibilidades de presentar cáncer de cuello uterino (Koliopoulos et al., 2017) (Kang et al., 2020)

En nuestra investigación se demuestra una buena correlación de kappa entre las muestras de auto toma y la muestra obtenida por el profesional: similares resultados fueron presentados por Swift et al 2020 en este estudio la concordancia alcanzó el 0,73 (Nutthachote et al., 2019). Esta situación refuerza la efectividad de los métodos de auto muestreo para el tamizaje primario del VPH.

Las técnicas de auto muestreo (en orina y vaginal) han demostrado una alta aceptabilidad en la población de áreas rurales (Rosenbaum et al., 2014), este tipo de métodos pude incrementar la adherencia al tamizaje en mujeres que tradicionalmente no acceden a exámenes médicos. Adicionalmente las técnicas de auto muestreo, puedes ser tomadas en lugares con poca infraestructura y ofertada por matronas, enfermeras o trabajadores de salud a nivel comunitario (Dutton et al., 2020).

Limitaciones del estudio.

Una limitación del estudio es que las participantes fueron seleccionadas de forma voluntaria de aquellas que aceptaron participar y se enmarcaban en los criterios de inclusión. Sin embargo, todas las participantes tienen similares características sociodemográficas haciéndolas comparables. Otra limitación es que un importante número de participantes tienen menos de 30 años lo cual puede incrementar la prevalencia de VPH en este estudio. Esto afecta los valores de VPP y VPN en nuestra muestra; típicamente los valores en poblaciones con baja prevalencia suelen ser mejores.

VIII. CONCLUSIONES

-Los métodos de auto toma vaginal y de orina, tienen similar sensibilidad y especificidad comparadas con la toma realizada por un profesional de salud para el diagnóstico del VPH.

-Los métodos de auto muestreo tienen una alta capacidad diagnóstica para la detección de casos positivos de VPH de AR.

-Los resultados positivos de VPH de BR y la ausencia de VPH en la muestra, tienen alta relevancia para la práctica clínica, pues, detectan o descartan el riesgo de cáncer cervical.

-Los resultados positivos de los genotipos de VPH de BR y la ausencia de VPH en tienen alta relevancia en la práctica clínica para detectar o descartar el riesgo de cáncer cervical

-Los métodos de automuestreo tienen alta sensibilidad y especificidad y han demostrado su eficacia en la zona rural

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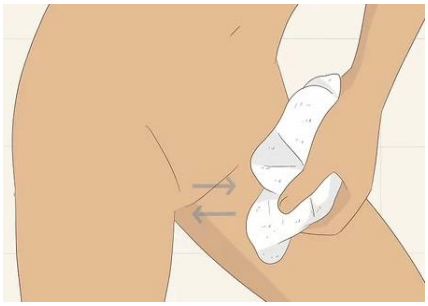
Anexo 1 Pictogramas para la toma de muestras

Técnica para toma del examen de orina (realizado por la paciente)

Lave sus manos con agua y jabón

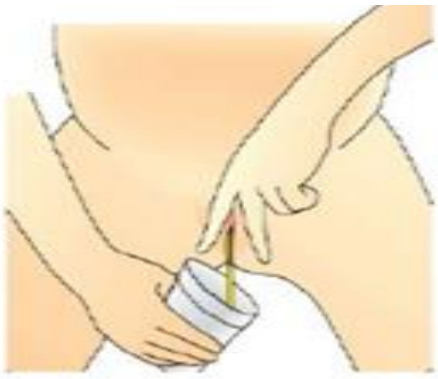
Limpie sus genitales con las toallas de limpieza



Retire el frasco de la envoltura y abra el frasco sin tocar el interior

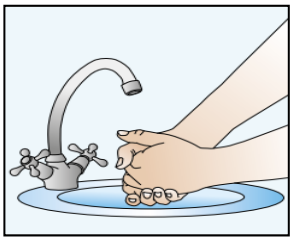
Separe los labios de la vagina y recolecte la orina

Coloque la tapa en el frasco recolector y ciérrelo

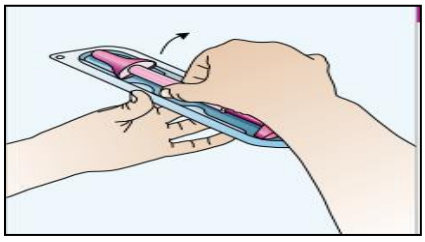


Técnica para Auto Toma (realizado por la paciente)

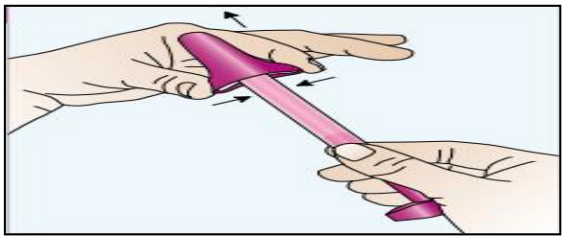
1 Lave sus manos con agua y jabón



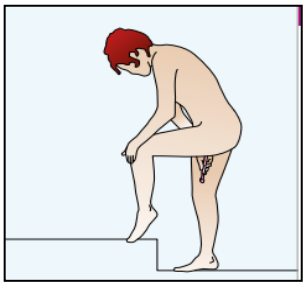
2 Retire el dispositivo de la envoltura y no tire la envoltura



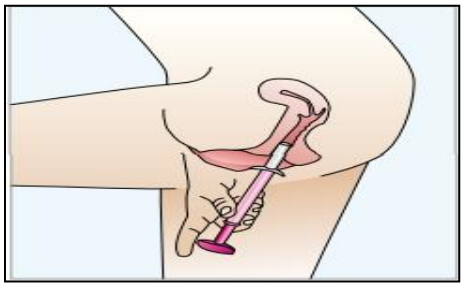
3 Presione los lados de la tapa rosada con sus dedos índice y pulgar y remueva la tapa



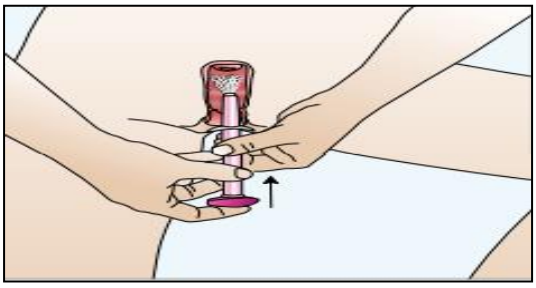
4 Colóquese en una posición cómoda levantado ligeramente la pierna



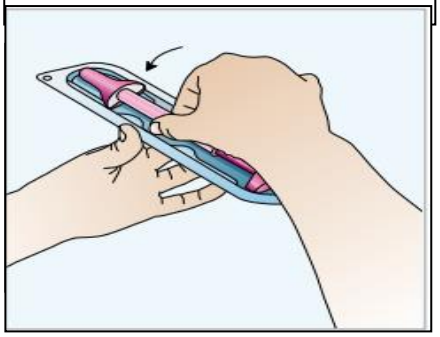
5 Separe los labios con una mano y con la otra introduzca el dispositivo en la vagina, hasta que los alerones toquen los labios



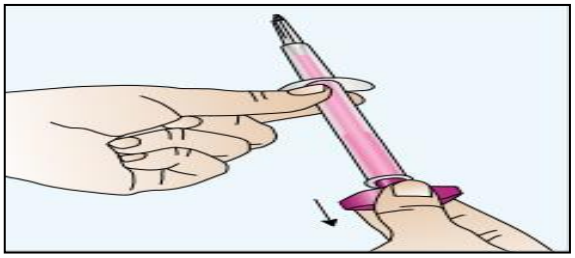
6 Sostenga el dispositivo con la una mano en el tubo trasparente y con la otra empuje el alerón rozados hacia la vagina hasta escuchar un click



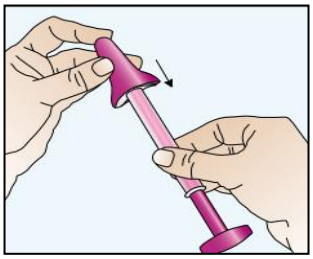
7 Gire el alerón rozado en una dirección por 5 veces, después de cada rotación usted escuchará un click



8 Retire el dispositivo de la vagina, sostenga con una mano el tubo trasparente y con la otra fraccione el alerón riscado hasta que el cepillo blanco desaparezca dentro del tubo



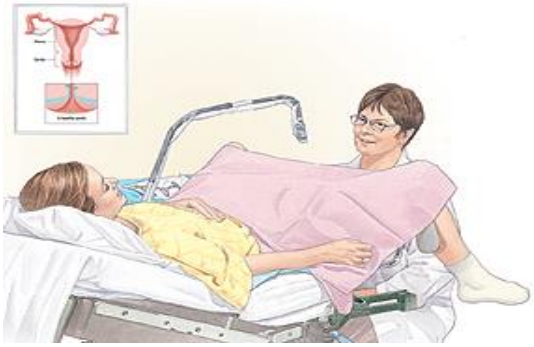
9 Coloque nuevamente la tapa sobre el dispositivo



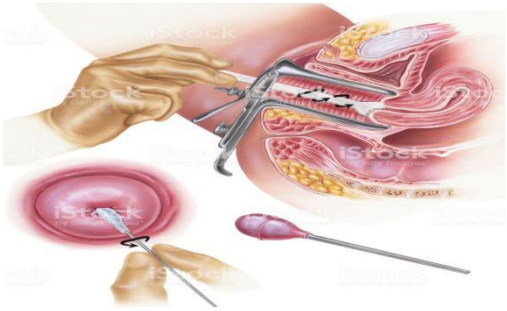
10 Coloque el dispositivo en la envoltura

Técnica de toma estándar con espéculo y cepillo (realizado por el médico o profesional de la salud)

1 El médico y médica le entregara una bata y le pedirá que se recueste sobre la mesa ginecológica como se puede observar en el gráfico



2 El médico y médica insertará un especulo suavemente en la vagina y tomará con un cepillo células que se encuentran en el cuello del útero



Anexo 2

PROTOCOLO DE EXTRACCIÓN DE ADN, PCR E HIBRIDACIÓN POR FLUJO DIRECTO UTILIZANDO EL KIT 37HPV GENOARRAY HBGA-37PKG HYBRIBIO

1. EXTRACCIÓN DE ADN VIRAL

El siguiente protocolo se utiliza para la extracción de material genético viral de las muestras de citología cervical (frotis cervical y auto toma) y las muestras de orina utilizando el kit: **HYBRIBIO CELL LYSIS KIT**

Objetivo: Extraer el material genético viral (ADN) de muestras de cepillado cervical a partir de solventes orgánicos.

Almacenamiento y estabilidad: El kit debe almacenarse a temperatura ambiente lejos de la luz solar. En estas condiciones los reactivos se mantienen estables por 12 meses.

Requerimientos:

a) Materiales
- Tubos eppendorf 1.5mL con tapón de rosca
- Puntas de pipeta automática de 1000µl
- Puntas de pipeta automática de 10µl
- Guantes de nitrilo

b) Reactivos
- Hybriobio Cell Lysis Kit

c) Equipos
- Microcentrífuga que alcance las 14000rpm
- Vórtex
- Baño maría
- Pipetas automáticas de 10µl y 100µl

Procedimiento:

1) Pipetear 1 ml de muestra cervical en un tubo de micro-centrífuga de 1.5 ml o en un tubo de tapón de rosca.
2) Centrifugar a 14.000m rpm durante 5 minutos (para aquellos que usan tubos con tapón de rosca, coloque una marca en el tapón del tubo y la marca debe quedar hacia afuera durante la centrifugación).
3) Desechar el sobrenadante.
4) Re-suspender el sedimento en 400 ul de la **Solución 1**, agitar en vórtex con intensidad, luego hervir en un baño de agua caliente (95 ° C o más) durante 15 minutos, dejar enfriar el tubo durante 2 minutos.
5) Agregar 400 ul de la **Solución 2**, mezclar suavemente, esperar 2 minutos a temperatura ambiente.
6) Centrifugar el tubo a 14.000 rpm durante 5 min. (la marca debe quedar hacia afuera para el tubo con tapón de rosca), luego desechar el sobrenadante.
7) Centrifugue el tubo nuevamente a 14.000 por un minuto, luego use una punta de micro-pipeta para pipetear el resto del solvente en el tubo.

8) Dejar reposar el tubo durante 2 minutos a temperatura ambiente.
9) Añadir 30 µl de la **Solución 3** al tubo, agitar en vórtex durante 10 segundos, centrifugar por 10 segundos, luego pipetear 1µl de la solución tomando una muestra de la mitad superior del volumen para la amplificación por PCR. (Nota: No pipetear la base del tubo).

2. REACCIÓN EN CADENA DE LA POLIMERASA

El siguiente protocolo se utiliza para la amplificación del material genético purificado con el procedimiento previo utilizando: **KIT 37HPV GENOARRAY HBGA-37PKG.**

Objetivo: Amplificar el material genético viral mediante PCR utilizando cebadores específicos para 37 genotipos diferentes del virus de papiloma humano que se muestran a continuación:

Alto riesgo	16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66,68
Bajo riesgo	6, 11, 42, 43, 44, CP8304 (81)
Riesgo indeterminado	26, 34, 40, 54, 55, 57, 61, 67, 69, 70, 71, 72, 73, 82, 83, 84

Almacenamiento y estabilidad: El kit debe almacenarse en una zona pre-PCR, nunca debe almacenarse en una zona post-PCR. Los reactivos deben almacenarse en una temperatura de 2-8ºC evitando ciclos de congelación – descongelación.

Requerimientos:

a) Materiales
- Guantes de nitrilo
- Tubos eppendorf 1.5mL
- Tubos para PCR de 0.2mL tapa plana
- Puntas de pipeta automática de 1000µl
- Puntas de pipeta automática de 100µl
- Puntas de pipeta automática de 10µ

b) Reactivos
- Etanol al 70%
- Agua destilada libre de DNAsa.
- Kit 37HPV GENOARRAY HBGA-37PKG.

c) Equipos
- Termociclador
- Minicentrífuga para tubos de 0.2 a 1.5mL

Antes de empezar, limpiar toda la zona de trabajo con etanol al 70% incluyendo las pipetas.

El volumen total de reacción de PCR es de 25µl con la siguiente composición

Componentes	Volumen de reacción
Mezcla de PCR (tapa azul)	23.25 µl
DNA Taq Polimerasa	0.75 µl
Molde de DNA	1 µl

Procedimiento:

1) Descongelar la mezcla de PCR y el agua libre de DNAsa a temperatura ambiente.
2) Agitar y centrifugar los reactivos a 8000rpm por 1 minuto antes de usar.
3) Centrifugar la DNA Taq polimerasa 8000rpm por 30 segundos.
4) Descongelar el DNA y centrifugar a 8000rpm por 5 minutos.
5) Preparar la mezcla utilizando los volúmenes de la tabla anterior, agitar y centrifugar la mezcla.

NOTA: La DNA Taq polimerasa debe ser sacada del congelador justo antes de su uso y debe guardarse inmediatamente.

6) Distribuir alícuotas de 24µl de la mezcla en cada tubo de PCR de 0.2mL.
7) Añadir 1µl de la muestra de DNA a cada tubo de reacción.
8) Centrifugar las muestras suavemente.
9) Colocar los tubos en el termociclador e iniciar el programa de amplificación con los siguientes parámetros:

	Temperatura	Tiempo
Desnaturalización	95ºC	9 minutos
40 ciclos		
Desnaturalización	95ºC	20 segundos
Hibridación	55ºC	30 segundos
Elongación	72ºC	30 segundos
Extensión final	72ºC	5 minutos
Incubación	4ºC	∞

3. HIBRIDACIÓN POR FLUJO DIRECTO

El siguiente protocolo se utiliza para la hibridación por flujo directo a partir de los productos de la PCR, utilizando una membrana de nitrocelulosa contenida en el **Kit 37HPV GENOARRAY HBGA-37PKG.**

Objetivo: Evidenciar si se produjo o no una amplificación del genoma viral de uno o más genotipos del virus de papiloma humano.

Almacenamiento y estabilidad: Los soluciones de hibridación deben almacenarse a temperatura de 4-8ºC, en estas condiciones son estables por 12 meses. La solución NBT/BCIP es sensible a la luz por lo que es necesario protegerla en un envase marrón. Después de utilizarse, la solución debe guardarse a 4ºC.

Requerimientos:

a) Materiales
- Puntas de pipeta automática de 1000µl
- Puntas de pipeta automática de 100µl
- Guantes de nitrilo
- Hielo
- Pinzas

b) Reactivos
- Kit de hibridación 37HPV GENOARRAY HBGA-37PKG.

c) Equipos
- Termobloque
- HybriMax
- Baño maría
- Pipeta automática de 100µl
- Pipeta automática de 1000µl

Preparación antes del experimento:

1) Precalentar la **Solución de Hibridación (HYB SOLN)** en baño de agua a 45ºC antes de usar. Todo el resto de soluciones deben estar a temperatura ambiente.
2) Si en la **Solución B (SOLN B)** se forma un precipitado, disolver incubando en baño de agua a 45ºC y dejando que llegue posteriormente a temperatura ambiente.
3) Utilizar el frasco marrón para preparar la solución de trabajando a partir de disolver el comprimido **NBT/BCIP** en 10mL de **Solución C.**
4) Desnaturalizar los productos de PCR calentándolos a 95ºC por 5 minutos y luego producir un choque térmico colocándolos en hielo por 2 minutos.

Procedimiento:

1) Instalar todos los componentes del **HybriMax** en el dispositivo **HybriMax**.
2) Ajustar la temperatura del **HybriMax** a 45ºC.
3) Cuando la temperatura alcance los 45ºC, añadir 0.8mL de **Solución de Hibridación** (precalentada a 45ºC) en los pocillos donde se encuentran las membranas **"HybriMem"** durante 3 minutos.
4) Extraer todo el solvente con succión de la bomba.
5) Añadir 0.5mL de **Solución de Hibridación** (precalentada a 45ºC) en los pocillos donde se encuentran las membranas **"HybriMem"**.

6) Agregar en los pocillos, las muestras amplificadas de DNA desnaturalizados (una por pocillo), pipetear 2 a 3 veces cada una para mezclar la solución cuidadosamente.
7) Cerrar la tapa del **HybriMax**.
8) Incubar durante 20 minutos.
9) Extraer todo el solvente con succión de la bomba.
10) Agregar 0.8mL de **Solución de Hibridación** (precalentada a 45ºC).
11) Repetir los pasos 9 y 10 dos veces más.
12) Apagar la bomba de succión.
13) Fijar la temperatura del **HybriMax** a 25ºC.
14) Cuando la temperatura alcance los 30ºC, agregar 0,5mL de **Solución de Bloqueo** en cada pocillo (la temperatura está descendiendo hasta alcanzar los 25ºC).
15) Extraer todo el solvente con succión de la bomba.
16) Nuevamente, agregar 0,5mL de **Solución de Bloqueo** en cada pocillo e incubar por 5 minutos. Extraer todo el solvente con succión de la bomba.
17) Cuando la temperatura del HybriMax alcance los 25ºC, agregar 0,5mL de **Conjugado Enzimático,** e incubar por 3.5 minutos.
18) Extraer todo el solvente con succión de la bomba.
19) Añadir 0,8mL de **Solución A** (lavado).
20) Repetir los pasos 18 y 19 tres veces más.
21) Apagar la bomba.
22) Fijar la temperatura del **HybriMax** a 36ºC.
23) Cuando la temperatura alcance lo 36ºC, agregar 0,5mL de **Solución NBT/BCIP** e incubar por 5 minutos. La tapa del **HybriMax** debe estar cerrada.
24) Extraer todo el solvente con succión de la bomba.
25) Mantener la bomba encendida.
26) Añadir 0.8mL de **Solución B** (lavado).
27) Repetir el paso 26 dos veces más.
28) Añadir 1mL de **agua destilada** (enguaje).
29) Apagar la bomba.
30) Retirar la tapa de fijación y todos los accesorio. Utilizar pinzas para sacar las membranas y secar en papel absorbente.
31) Interpretar los resultados por visualización.

NOTA: no tocar las membranas con las manos/dedos sin guantes. Usar siempre guantes y pinzas.

Limpiar el área de trabajo antes y después de la prueba con alcohol al 70% e hipoclorito de sodio al 10%.

Interpretación de los resultados

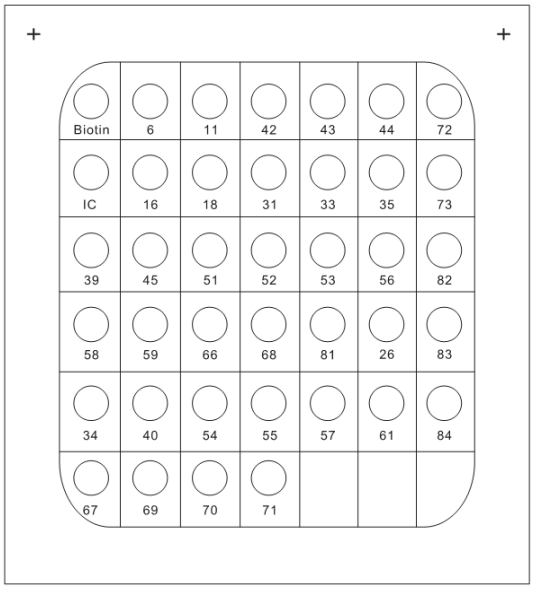


Figura No. 1 Diagrama esquemático de la membrana diagnóstica del 37HPV GenoArray HBGA-37PKG

Claves:

BIO: Control de biotina

IC: Control interno

Números: Corresponden a cada genotipo específico de VPH.

:

COMPARACIÓN DE LA EFECTIVIDAD DE LAS PRUEBAS DE AUTO MUESTREO PARA EL DIAGNÓSTICO DEL VIRUS DEL PAPILOMA HUMANO

Esta tesis doctoral ha generado los siguientes artículos científicos y aportaciones a congresos:

Titulo:
Role of Self-Sampling for Cervical Cancer Screening: Diagnostic Test Properties of Three Tests for the Diagnosis of HPV in Rural Communities of Cuenca, Ecuador
Publicada en:
International Journal of Environmental Research and Public Health 2022, 19, 4619. https://doi.org/10.3390/ijerph19084619
Autores:
Bernardo Vega Crespo, Vivian Alejandra Neira, José Ortíz Segarra, Ruth Maldonado Rengel, Diana López , María Paz Orellana, Andrea Gómez , María José Vicuña , Jorge Mejía , Ina Benoy, Tesifón Parrón Carreño and Veronique Verhoeven.

Título:

Universal cervical cancer control through a right to health lens: refocusing national policy and programmes on underserved women
Publicado en:
BMC International Health and Human Rights (2020) 20:21 https://doi.org/10.1186/s12914-020-00237-9
Autores :
Katrina Perehudoff, Heleen Vermandere, Alex Williams, Sergio Bautista-Arredondo, Elien De Paepe, Sonia Dias, Ana Gama3,5, Ines Keygnaert, Adhemar Longatto-Filho, Jose Ortiz11, Elizaveta Padalko, Rui Manuel Reis, Nathalie Vanderheijden, Bernardo Vega, Bo Verberckmoes, and Olivier Degomme

Aportaciones a congresos

Titulo
"Role of Self-Sampling for the Diagnosis of Human Papillomavirus in Rural Areas from Cuenca, Ecuador: Comparison of Acceptance, Sensitivity and Specificity among Urine Sampling, Self-Sampling and Clinician Sampling**"**
Tipo de aportación: Poster
Fecha: 23 and 24 de septiembre de 2021
Lugar: Ohio, Estados Unidos
Universidad: Candase Western Reserve University School of Medicine USA
Reconocimiento. Best poster award

Nombre del congreso*:* Affordable Cancer Technologies (ACT)
Autores:
Bernardo Vega C, Vivian Alejandra Neira, Ruth Maldonado-Rengel, Diana López, María Paz Orellana, Santiago Dávila, María Angélica Morales, Ina Benoy, Tesifón Parrón Carreño Veronique Verhoeven

Titulo
"Aceptabilidad y sensibilidad del rol del auto muestreo para el diagnóstico del virus del papiloma humano**"**
Tipo de aportación: Presentación oral
Fecha: 15 al 18 de noviembre de 2021
Lugar: Riobamba, Ecuador
Universidad: Universidad Técnica de Ambato
Nombre del congreso*:* VIII Congreso internacional de investigación REDU
Autores:
Bernardo Vega C, Vivian Alejandra Neira, Ruth Maldonado-Rengel, Diana López, María Paz Orellana, Santiago Dávila, María Angélica Morales, Carreño, Ina Benoy, Tesifón Parrón, Veronique Verhoeven

Titulo:
"Aceptabilidad y sensibilidad del rol del auto muestreo para el diagnóstico del virus del papiloma humano**"**
Tipo de aportación: Presentación oral
Fecha: 21 de octubre de 2021
Lugar: Guayaquil, Ecuador
Universidad: Escuela Politécnica del Litoral
Nombre del congreso*:* "Workshop: Multi-omic approaches for nutritional health monitoring"
Autores

Bernardo Vega C, Vivian Alejandra Neira, Ruth Maldonado-Rengel, Diana López, María Paz Orellana, Santiago Dávila, María Angélica Morales, Carreño, Ina Benoy, Tesifón Parrón, Veronique Verhoeven

Titulo

“Aceptabilidad y sensibilidad del rol del auto muestreo para el diagnóstico del virus del papiloma humano**”**
Tipo de aportación: Presentación oral
Fecha: 20 al 17 de septiembre de 2021
Lugar: Cuenca, Ecuador
Universidad: Universidad de Cuenca
Nombre del congreso*:* I Congreso de investigación científica en salud materna, perinatal y neonatal
Autores:
Bernardo Vega C, Vivian Alejandra Neira, Ruth Maldonado-Rengel, Diana López, María Paz Orellana, Santiago Dávila, María Angélica Morales, Carreño, Ina Benoy, Tesifón Parrón, Veronique Verhoeven

International Journal of
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Article

Role of Self-Sampling for Cervical Cancer Screening: Diagnostic Test Properties of Three Tests for the Diagnosis of HPV in Rural Communities of Cuenca, Ecuador

Bernardo Vega Crespo [1,*], Vivian Alejandra Neira [1,2], José Ortíz Segarra [1], Ruth Maldonado Rengel [3,4], Diana López [2], María Paz Orellana [1], Andrea Gómez [1], María José Vicuña [1], Jorge Mejía [1], Ina Benoy [5], Tesifón Parrón Carreño [6] and Veronique Verhoeven [7]

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Abstract: Background: HPV primary screening has shown effectiveness for cancer prevention; however, gynaecological examination is considered uncomfortable. Self-sampling methods increase the acceptance of screening. The aim of this study is to compare the sensitivity and specificity of clinician sampling versus vaginal and urine self-sampling for HPV diagnosis. Methods: A diagnostic test study was conducted in a rural parish of Cuenca, Ecuador. A total of 120 women participated. Each participant self-collected urine and vaginal samples and underwent clinician sampling for HPV testing. The latter was considered as the golden standard. All three samples were processed with the same amplification and hybridization protocol for HPV detection (Hybribio) following the manufacturer's instructions. Results: Characteristics of the participants were: median age 35 years; 40.8% married; 46.7% had a primary level of education; and median age of sexual onset, 17.6 years. The prevalence of any type of HPV with clinician sampling was 15.0%, 17.5% with urine sampling and 18.3% with vaginal self-sampling. Self-sampling sensitivity reached 94.4% (IC 74.2–99.9), and specificity 92.1% (IC 85.2–95.9). Urine sampling had a sensitivity of 88.8% (IC 67.2, 96.9), and specificity 94.1% (IC 67.2–96.9). The negative predictive value was 98.9% (IC 94.2–99.8) for vaginal self-sampling and 97.6% (IC 92.6–99.4) for urine sampling. Conclusions: This study shows that vaginal and urine self-sampling methods have similar sensitivity and specificity compared with clinician sampling for the diagnosis of HPV. The correlation between HPV genotypes among the three tests is satisfactory.

Keywords: HPV; self-sampling; urine sampling; clinician sampling; sensitivity and specificity

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1. Introduction

Since the pap smear was implemented for population screening in 1970 for cervical cancer (CC) prevention, mortality has decreased by 70% worldwide [1]. However, the sensitivity of the conventional pap smear for the detection of cellular abnormalities varies

between 51% and 55%, and the specificity is between 66.6% and 75% [2,3]. Due to those rates, the success of this test depends on the adherence to repeated screening during a woman's lifetime in order to find precancerous lesions in early stages. To reach screening objectives, it is recommended that the intervals between pap smears last no longer than 3 years [4].

The detection of human papilloma virus (HPV) used as a primary screening method has a higher sensitivity (95%) and specificity (94% to 95%) than cytology to detect premalignant lesions [5–7]. A negative HPV test could extend the intervals of screening to five years according to some protocols [5,8]. The global strategy for cervical cancer prevention of the World Health Organization (WHO) considers that CC mortality could effectively be prevented if every woman has at least two high-sensitivity cervical screening episodes during their lifetime (at 35 and 45 years old) [9].

Screening and vaccination against HPV are reducing CC mortality in countries with strong preventative programs [10]. However, in low- and middle-income countries (LMIC), barriers to cervical cancer screening persist [11]. The rates of under-screened women are variable among countries. In Ecuador, 41.6% of women of reproductive age have never been screened [12].

Several barriers have been identified for access to cervical CC screening services; according to the socio-ecological model, they interact at different levels: organizational (difficulties in access to health centres and long waiting times), interpersonal (stigma and lack of family support) and personal (lack of risk perception and fear of examination) [13–15]. Furthermore, the COVID-19 pandemic has increased the disparities in access to healthcare facilities [16].

Self-sampling methods, such as vaginal self-sampling and urine sampling for HPV diagnosis, have demonstrated high acceptability and sensitivity for cervical cancer screening [17,18]. Compared with clinician sampling, they are less invasive and could be more attractive to increase the uptake in under-screened and never-screened women and to overcome barriers at different levels of interaction [18,19].

On the other hand, doubts about the sensitivity of self-sampling tests could discourage women to choose those tests. There are limited studies that compare the sensitivity and specificity of vaginal self-sampling and urine sampling for HPV detection at the same time and in the same population.

A literature review reflects a wide range of variation in sensitivity and specificity among tests and techniques [20–26].

Type of Sample	Sensitivity	Specificity	Author
Vaginal self-sampling	83.3%	73.9%	Asciutto et al., 2018
Vaginal self-sampling	84%	93%	Arbyn et al., 2018
Vaginal self-sampling	84.6%	62.9%	Wang et al., 2020
Vaginal self-sampling	50%	98%	Esber et al., 2018
Vaginal self-sampling	98.9%	100%	Kuriakos et al., 2019
Urine self-sampling	48.1%	82.8%	Asciutto et al., 2018
Urine self-sampling	90.5%	74.0%	Combita et al., 2016

This is the first study of self-sampling effectiveness conducted in Ecuador; the aim of this research is to compare sensitivity, specificity, predictive values and correlation for the diagnosis of HPV from vaginal self-sampling and urine self-sampling versus clinician sampling in a rural setting.

2. Materials and Methods

2.1. Ethical Statement

This study was approved under the guidance of the Declaration of Helsinki and the Council for International Organizations of Medical Sciences (CIOMS). All procedures involving human participants were approved by the bioethical committee of the University of Cuenca (approval code UC-COBIAS-2020-262) and the National Directory of Intelligence

in Health (DIS) of the Ministry of Health of Ecuador (approval code MSP-DIS-2020-0405-O). All the participants were informed about the purpose of the study and signed an informed consent form before the sample collection.

2.2. Study Population

A diagnostic test study was conducted in the rural parish of El Valle of Cuenca Ecuador from May to August 2021. Through flyers delivered in public places, households and in healthcare centres, women of El Valle were invited to participate.

The inclusion criteria included being sexually active; being between 18 and 70 years old; not having undergone an excision or destructive treatment of the cervical intraepithelial neoplasm; not having used vaginal medication at least a week before the examination; not having had sexual intercourse for at least 48 h previous to the examination; not being pregnant; and the absence of menstrual bleeding at the time of examination. One hundred twenty women participated and provided three samples: urine, a vaginal sample and a physician-taken cervical assay.

2.3. Sampling Collection

Prior to sample collection, patients who agreed to participate were taught how to take the samples: a pictographic representation of each technique was given to all participants and the same graphics were present in the bathroom where the patients were instructed to obtain the samples.

The first sample obtained was a urine sample; it was obtained directly in a sterile urine container after self-made asepsis by the patient in the bathroom of the consultation room. All patients were asked to collect at least 30 cc of urine.

After urine sampling, a self-sampling device for vaginal sampling was given to the patient. An Evalyn® Brush from Rovers Medical Devices was the selected tool for this sampling. Fabricant instructions were used for sample collection; researchers waited outside of the bathroom for any additional explanation and to receive the sample after its collection. Finally, the patient was directed to a gynaecological examination table. After the insertion of the speculum, endocervix and exocervix samples were obtained by using a Hybribio cervical brush, rotating 360° twice. The cervical brush was placed in Roche Cell Collection Medium for transportation. This medium was selected because it contains 20 cc of preservant, which allows the researcher to centrifuge and obtain material for HPV diagnosis.

All the samples were paired, labelled, coded and transported to the laboratory of molecular biology of the University of Cuenca within the first 6 h of collection. The results for HPV were delivered to the patients within 10 days after the sample collection.

2.4. HPV Genotyping

Once in the laboratory, the 3 samples (clinician sampling, self-sampling, and urine sampling) from each patient were processed to obtain the genetic material. For urine DNA purification, we used the DNA prep kit (Hybribio, Guangzhou, China) following the manufacturer's instructions. For the conventional collection samples, we used the Cell Lysis Kit (Hybribio) following the manufacturer's protocol. Finally, the self-sampling brushes were washed for about 1 min in Hybribio Female Sample Collection media to release the cells and purify the genetic material using the same extraction kit as the conventional collection. All the material obtained was stored at −20 °C until further use.

For amplification, we used the 37 HPV GENOARRAY kit (Hybribio), which allows the simultaneous amplification of 37 different genotypes, including high-risk genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, and 68; and the low-risk (LR) ones: 6, 11, 42, 43, 44, and CP8304 (81); and 26, 34, 40, 54, 55, 57, 61, 67, 69, 70, 71, 72, 73, 82, 83, 84 categorized as undetermined risk. The PCR mix was performed according to the manufacturer's instructions to obtain a final reaction volume of 25 µL (23.25 µL PCR Mix, 0.75 µL of DNA Taq polymerase 5 U/µL and 1 µL of DNA). For urine samples, the final

volume was 26 µL because we added 2 µL of DNA template. Amplification was performed in the Veriti Thermal Cycler (Applied Biosystems, Waltham, MA, USA) with the following programming: initial denaturation at 95 °C for 5 min, 40 cycles of denaturation at 95 °C for 20 s, annealing at 55 °C for 30 s and elongation at 72 °C for 30 s, to finish with a final elongation at 72 °C for 5 min.

Finally, all the amplicons were denatured for 5 min at 95 °C and placed on ice before continuing the hybridization. The process was carried out in the HibriMax (Hybribio) according to the manufacturer's instructions. We used HPV-37 Hybrimem membranes containing the immobilized probes of the genotypes of interest. Streptavidin–horseradish peroxidase conjugate was added to bind to the biotinylated PCR products. The direct visualization of the breakdown product (purple precipitate) of the substrate nitroblue tetrazolium-5-bromo-4-chloro3-indolylphosphate was interpreted as positive for the corresponding HPV DNA type as indicated on the schematic diagram of the membrane provided with the test kit.

2.5. Data Analysis

Completed questionaries with sociodemographic data and the results of HPV tests were transcribed to a Microsoft Excel 2016 spreadsheet for cleaning and coding and were subsequently transferred to the Statistical Package for the Social Sciences for Windows version 17.0 (IBM, Armonk, NY, USA). Descriptive statistics were presented using means and standard deviation (SD) for continuous variables and percentages for categorical variables. Open-Source Epidemiologic Statistics for Public Health (Rollins School of Public Health de la Universidad de Emory, Atlanta, GA, USA) was used to calculate sensitivity, specificity, positive predictive value, negative predictive value, likelihood positive ratio, likelihood and Cohen's kappa. The kappa statistic was calculated to determine the level of chance agreement between the two methods, with a kappa value of 0 indicating no agreement better than chance, 1 indicating perfect agreement better than chance, and intermediate values of 0.00–0.20, 0.21–0.40, 0.41–0.60, 0.61–0.80 and >0.81 indicating poor, fair, moderate, good and excellent agreement.

Clinician cervical sampling with a speculum is the standard method for the diagnosis of HPV; therefore, the sensitivity and specificity of HPV detection in urine and vaginal self-sample were calculated using cervical sampling as reference. The width of the confidence intervals of kappa and the diagnostic accuracy parameters show the precision of our estimates.

3. Results

3.1. Population Characteristics

A total of 120 women participated in this study, all of them living in the rural parish. The sociodemographic characteristics of the participants are shown in Table 1.

Table 1. Participants' socio-demographic characteristics.

Variable	N (%)
Age: mean 35; mode 24; SD ± 11.23	
19 to 29	42 (35.5)
30 to 39	32 (26.7)
40 to 49	31 (25.8)
50 to 59	12 (10.8)
60 to 69	2 (1.7)
Educational level	
None	8 (6.7)
Alphabetization centre	1 (0.8)
Primary School	56 (46.7)
High school	43 (35.8)
University	11 (9.2)
Post graduate	1 (0.8)

Table 1. *Cont.*

Variable	N (%)
Civil status	
Married	49 (40.8)
Living as a couple	28 (23.3)
Single	25 (20.8)
Divorced	11 (9.2)
Separated	3 (2.5)
Widow	4 (3.3)
Occupation	
Housewife	69 (57.5)
Employed	27 (22.5)
Agriculture	3 (2.5)
Student	2 (1.7)
Retired	1 (0.8)
Stylist	1 (0.8)
Seller	1 (0.8)
Cleaning	1 (0.8)
Others	3 (2.5)
Family Income (USD)	
<100	22 (18.3)
100 to 200	21 (17.5)
201 to 300	19 (15.8)
301 to 400	23 (19.2)
401 to 500	17 (14.2)
501 to 600	6 (5.0)
>600	12 (10.0)
Age of sexual onset: median 17.6; mode 18; SD ± 2.9	
9 to 14 years	12 (10.0)
15 to 19 years	82 (68.3)
20 to 24 years	23 (19.2)
25 to 29 years	2 (1.7)
30 to 34 years	1 (0.8)
Previous cervical screening	
Yes	98 (81.7)
No	22 (18.3)

3.2. Comparison of Tests

The prevalence of any type of HPV with clinician sampling was 15.0%, 17.5% with urine self-sampling and 18.3% with vaginal self-sampling.

Table 2 presents the most prevalent HPV genotypes found in the three sampling techniques: 58, 51, 31, 52, 53, and 16. The following genotypes were detected in self-sampling but not in clinician sampling: 11, 33, 68, and 72. On the other hand, 11, 54, 68, and 73 were detected in urine sampling and not in clinician sampling.

Table 2. Distribution of any type of HPV according to sampling method.

Genotype	11 -	16 *	18 *	31 *	33 *	39 *	51 *	52 *	53 **	54-	56 *	58 *	66 **	68 *	70 -	71 -	72 -	73 *	81 -	84 -
Clinician sampling	-	1 (4.3)	1 (4.3)	3 (13.0)	-	1 (4.3)	3 (13.0)	2 (8.7)	2 (8.7)	-	1 (4.3)	4 (17.4)	1 (4.3)	-	1 (4.3)	1 (4.3)	-	-	1 (4.3)	1 (4.3)
Self-sampling	1 (3.2)	2 (6.5)	1 (6.5)	3 (9.7)	1 (3.2)	1 (3.2)	4 (12.9)	4 (12.9)	2 (6.5)	-	1 (3.2)	5 (19.4)	1 (3.2)	1 (3.2)	1 (3.2)	1 (3.2)	1 (3.2)	-	-	1 (3.2)
Urine sampling	1 (3.4)	2 (6.9)	1 (6.9)	3 (10.3)	-	1 (3.4)	4 (13.8)	1 (3.4)	2 (6.9)	2 (6.9)	1 (3.4)	4 (13.8)	2 (6.9)	1 (3.4)	1 (3.4)	-	-	1 (3.4)	1 (3.4)	1 (3.4)

* High-risk HPV. ** Middle-risk HPV. - Low-risk HPV.

Table 3 shows the comparison among the tests: the self-sampling sensitivity reached 94.4% (IC 74.2–99); specificity, 92.1% (IC 85.2–95.9); predictive positive value (PPV), 68.0% (IC 48.4–82.8); predictive negative value (PNV), 98.9% (IC 94.28, 99.81); positive likelihood ratio (PLR), 12 (IC 9.36–15.49); and negative likelihood ratio (NLR), 0.06 (IC 0.008–0.428). Agreement with clinician sampling was 0.74 (kappa).

The urine sampling had a sensitivity of 88.8% (IC 67.2, 96.9); specificity of 94.1% (IC 87.76, 97.28); PPV of 72.2% (IC 51.85, 86.85); PNV of 97.6% (IC 51.85–86.85); PLR of 15 (IC 10.73–21.27); and NLR 0.11 (IC 0.04–0.315). The agreement with clinician sampling was 0.76 (kappa).

Table 3. Comparison of sensitivity, specificity, predictive values, likelihood ratio and correlation among three tests.

		Clinician Sampling		Sensitivity	Specificity	PPV	PNV	LR+	LR−	Kappa
	Result	**Positive** ***n*** **(%)**	**Negative** ***n*** **(%)**	**%** **(IC 95%)**	**%** **(IC 95%)**	**%** **(IC 95%)**	**%** **(IC 95%)**	***n*** **(IC)**	***n*** **(IC)**	***n*** **(IC)**
Self-sampling	Positive	17 (14.2)	8 (6.7)	94.4 (74.2–99.0)	92.1 (85.2–95.9)	68.0 (48.4–82.8)	98.9 (94.28, 99.81)	12.0 (9.361–15.49)	0.06 (0.008–0.428)	0.74 (0.57–0.92)
	Negative	1 (0.8)	94 (78.3)							
Urine sampling	Positive	16 (13.3)	6 (5.0)	88.8 (67.2, 96.9)	94.1 (87.76, 97.28)	72.7 (51.85, 86.85)	97.6 (92.86, 99.44)	15.1 (10.73–21.27)	0.11 (0.044–0.315)	0.76 (0.58–0.93)
	Negative	2 (1.7)	96 (80.0)							

4. Discussion

This study demonstrates that urine self-sampling and vaginal self-sampling have similar sensitivity and specificity to clinician sampling for the diagnosis of HPV.

The general prevalence of HR and LR HPV genotypes is highly variable in the literature. In Europe, the range of HPV positivity goes from 2% in Spain to 12% in Belgium [27]. In Ecuador, this variation is also present. Cabrera J. et al. in 2015 reported a prevalence of 25.6% in Cuenca, in the Azuay province of Ecuador [28]. González-Andrade F. et al. in 2019 reported a prevalence of 6.3% among mestizo women [29]. Our findings (15%) are similar to the results presented by Dunne E. et al. in the United States, showing a prevalence of 17% [30]. A possible explanation for the high prevalence of HPV in a rural population is the large number of women below 30 years included in the study [31].

The prevalence of any type of HPV was slightly higher in vaginal self-sampling (18.3%) and urine sampling (17.5%); similar results were found by Polman N. et al. in 2019 in the Netherlands, where the HPV positivity rate with self-sampling was 7.4% vs. 7.2% with clinician sampling [32]. In urine and vaginal self-sampling, a higher number of cells could be recovered from the genital tract when compared with clinician sampling, which only collects endo- and exocervical cells. That could explain the higher rates of positivity in self-sampling methods.

HPV genotype also presents variation worldwide. In Europe, the most prevalent genotypes are 16, 18, 31, and 33 [27], and in China, they are 52, 58, 31, 52, 39, and 68 [31]. Our study found similar results to those presented by González-Andrade F in Ecuador: 16, 18, 31, 52, 53, 56, and 58. This variation could be explained by the epidemiological prevalence of HPV according to the area where the participants involved in each study reside [29].

The values of the sensitivity and specificity of vaginal self-sampling are comparable to the findings of Arbyn et al., 2018 (84% and 93%) [21], and are lower than the results from Kuriakos et al., 2019 (98.9% and 100%) (26). The standardization of the technique could explain this variation. However, this method has demonstrated equal efficiency to clinician sampling in large studies [32].

For urine sampling, our results are similar to the sensitivity and specificity reported by Combita et al. in 2016 (90.5–74.0%) [22]. However, our research sensitivity was higher. This difference could also be explained by the employed technique: this research used a reactive substance designed specifically for urine, which could increase the effectiveness.

A pap smear has a mean sensitivity of 51% [2]. Both methods, urine and vaginal self-sampling, could be considered more efficient because their sensitivity is higher than

80% [33]. Screening would be equally reliable and could be less frequent than pap smears. In addition, the high specificity and negative predictive value are relevant for clinical practice, because patients with negative results rarely present a cervical lesion and therefore have low risk for cervical cancer [6,34].

Our research reported a good kappa correlation with clinician sampling; similar results are reported by Swift et al., 2020, with agreement reaching 0.73 [35]. This situation reinforces the effectiveness of self-sampling methods for the primary screening of HPV.

Self-sampling methods (urine and vaginal sampling) have a high acceptability among populations in rural areas [36], which could increase the uptake of examination. In addition, self-samples may be collected in low-infrastructure settings and be offered by midwives and healthcare workers at the community level [37].

Limitations

A limitation of the study is that participants were selected by convenience, and all patients who agreed to participate and met the study criteria were included in the study. However, women that participated had similar sociodemographic characteristics, making them comparable. Another limitation is that an important number of women below 30 years old participated in the research, which could increase the prevalence rates of HPV in this study. This affects PPV and NPV in our sample; typically, in populations with a lower prevalence, NPV would be better.

Including young women was intentional in order to find more HPV-positive patients and to have a sufficient number of positive results to calculate sensitivity and specificity. Previous research conducted in Ecuador demonstrated a lower prevalence of HPV in rural areas compared with urban.

5. Conclusions

This research study is the first conducted in Ecuador evaluating the effectiveness of self-sampling methods in a rural community in Cuenca, Ecuador.

This study shows that vaginal and urine self-sampling methods have similar sensitivity and specificity to clinician sampling for the diagnosis of HPV. The Kappa correlation between HPV genotype among the three tests is good.

Self-sampling methods have a high diagnostic capacity and a capability for the detection of positive cases of HR HPV.

The positive results of LR HPV and absence of HPV have a high relevance in clinical practice for detecting or discarding risk for cervical cancer.

Self-sampling methods have high sensitivity and specificity and have demonstrated reliability in rural settings.

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Informed Consent Statement: Informed consent was obtained from all the participants. All personal information was encoded and treated confidentially.

Data Availability Statement: The datasets generated and/or analysed during the current study are not publicly available because they contain the sensitive personal information of participants. The informed consent grants the confidentiality of the participants' data. However, the datasets are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

CC	Cervical cancer
CAMIE	Cáncer Auto Muestreo Igualdad Empoderamiento/Cancer Self Sampling Equity Empowerment
DIS	National Directory of Intelligence in Health
DIUC	Dirección de investigación de la Universidad de Cuenca/Direction of Research of the University of Cuenca
HPV	Human papillomavirus
HR	High risk
LMIC	Low- and middle-income countries
LR	Low risk
UC-COBIAS	University de Cuenca Comité de Bioética de las áreas de la Salud/University of Cuenca Comitee of Bioethics of Health Science Areas
VLIR-UOS	Vlaamse Interuniversitaire Raad Universitaire Ontwikkelingssamenwerking (Flemish Interuniversities Council University Development Co-operation)
WHO	World Health Organization

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DEBATE

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Universal cervical cancer control through a right to health lens: refocusing national policy and programmes on underserved women

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Abstract

Background: Cervical cancer claims 311,000 lives annually, and 90% of these deaths occur in low- and middle-income countries. Cervical cancer is a highly preventable and treatable disease, if detected through screening at an early stage. Governments have a responsibility to screen women for precancerous cervical lesions. Yet, national screening programmes overlook many poor women and those marginalised in society. Under-screened women (called hard-to-reach) experience a higher incidence of cervical cancer and elevated mortality rates compared to regularly-screened women. Such inequalities deprive hard-to-reach women of the full enjoyment of their right to sexual and reproductive health, as laid out in Article 12 of the International Covenant on Economic, Social and Cultural Rights and General Comment No. 22.

Discussion: This article argues first for tailored and innovative national cervical cancer screening programmes (NCSP) grounded in human rights law, to close the disparity between women who are afforded screening and those who are not. Second, acknowledging socioeconomic disparities requires governments to adopt and refine universal cancer control through NCSPs aligned with human rights duties, including to reach all eligible women. Commonly reported- and chronically under-addressed- screening disparities relate to the *availability* of sufficient health facilities and human resources (example from Kenya), the *physical accessibility* of health services for rural and remote populations (example from Brazil), and the *accessibility of information* sensitive to cultural, ethnic, and linguistic barriers (example from Ecuador). Third, governments can adopt new technologies to overcome individual and structural barriers to cervical cancer screening. National cervical cancer screening programmes should tailor screening methods to under-screened women, bearing in mind that eliminating systemic discrimination may require committing greater resources to traditionally neglected groups.

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Conclusion: Governments have human rights obligations to refocus screening policies and programmes on women who are disproportionately affected by discrimination that impairs their full enjoyment of the right to sexual and reproductive health. National cervical cancer screening programmes that keep the right to health principles (above) central will be able to expand screening among low-income, isolated and other marginalised populations, but also women in general, who, for a variety of reasons, do not visit healthcare providers for regular screenings.

Keywords: Cervical cancer, Human papillomavirus, Sexual and reproductive health, Right to health, Human rights, Cancer screening, Cancer prevention, National cancer policy, HPV test

Background

Cervical cancer is the fourth most common cancer in women, globally. Every two minutes a woman dies of cervical cancer around the world, resulting in 311,000 deaths annually [1]. Nine out of ten of these deaths occur in low- and middle-income countries (LMICs)- a fact that the World Health Organization (WHO) Director-General Dr. Tedros Adhanom Ghebreyesus labels 'neither fair nor just' [2]. *Why?* Cervical cancer is a highly preventable and treatable disease if detected at an early stage. Prevention and early detection is possible by checking for abnormalities in the cells of the cervix. Also the presence of the human papillomavirus (HPV) is an indication that one might be at risk for cervical cancer; especially the so called high-risk HPV types increase the risk of malignant lesions. Two of them, HPV 16 and 18, are found in over 70% of cervical cancer cases. These preventative measures have long hinged on a functioning health system, complete with trained gynaecologists, laboratory infrastructure, and vaccination and screening programs. All of these measures are necessary to work towards cervical cancer elimination, a global commitment in the 2013–2020 *Global Action Plan for the Prevention and Control of Noncommunicable Diseases*. The UN Population Fund has promised to support national health ministries in integrating this into existing reproductive health programmes [3].

Governments have a three-part responsibility in relation to cervical cancer over the course of a woman's life: during her youth, to vaccinate against HPV; in midlife to screen for precancerous cervical lesions; and at all ages to treat cancer, if needed [4]. These times are crucial thresholds that can become a life saved or a life lost. Of these three moments, regular screening and follow-up of *all* at-risk women is the pinnacle of cervical cancer control. When under-screened, women experience a higher incidence of cervical cancer and elevated mortality rates compared to regularly-screened women [5]. Yet, crucial shortcomings in national cervical cancer screening programmes (NCSPs) mean they overlook many poor women and those marginalised in society by their age, ethnicity, disability, language, place of residence, and/or recent immigration status, among other factors [6]. Such inequalities harm women twice, first by making them more vulnerable to acquiring HPV infections, and again by depriving them of potentially lifesaving screening and early cancer detection. Ultimately, these women cannot enjoy their right to health, as laid out in Article 12 of the International Covenant on Economic, Social and Cultural Rights (ICESCR). The 2016 General Comment No. 22 is an authoritative explanation of governments' obligations to realise the right to sexual and reproductive health (SRH) from Article 12 of the ICESCR.

At the core of this debate, is how NCSPs, especially those in LMICs, can effectively reach populations vulnerable to HPV infections through policy and practice. This article argues for tailored and innovative NCSPs grounded in human rights law to close the disparity between women who are afforded screening and those who are not. Human rights law has the potential to reorient social norms, political discourse, and government's legal obligations towards the needs of underserved women and girls [7]. Some NCSPs may be framed around human rights law, such as the *Kenyan National Cancer Control Strategy 2017–2022* mentioned below. However, to the authors' knowledge, no systematic study of NCSPs' alignment with human rights law has been undertaken. Human rights-based screening programmes have the potential to make an important contribution to attaining, by 2030, a one-third reduction in premature mortality from non-communicable diseases (NCDs), such as cervical cancer (Sustainable Development Goal Target 3.4).

In the following sections of this paper, we first examine States' human rights obligations towards cervical cancer control, including through NCSPs, as outlined in General Comment No. 22, and the need to address disparities. We then recommend that acknowledging socioeconomic disparities requires governments to adopt and refine universal cancer control through NCSPs aligned with human rights duties, including to reach all eligible women. Third, we advocate for governments in countries with disparities in cervical cancer incidence and mortality to adopt new technologies to overcome

individual and structural barriers to cervical cancer screening.

Discussion

Cervical cancer control as part of the right to sexual and reproductive health

The right to the highest attainable standard of physical and mental health ('right to health') is embedded in numerous international treaties, including the most prominent, the ICESCR. In total, 169 national governments (or States) have ratified the ICESCR, and are therefore, legally obliged to realise the right to health. Due to ongoing and grave violations of people's SRH, the UN Committee on Economic, Social and Cultural Rights (UN CESCR) drew attention to the 'right to sexual and reproductive health' ('right to SRH'), a component of the right to health, in General Comment No. 22 (2016) [4]. This is a non-binding, yet highly authoritative, explanation of government obligations to realise the right to SRH.

Each State must use its own means and methods to realise the right to SRH with a maximum of its available resources, according to General Comment No. 22. This flexibility recognises that there is no 'one-size-fits-all' approach to SRH; instead, each government should use a tailored strategy to respond to local SRH needs and challenges within its own resources. Despite this flexibility, each State is under the immediate obligation "to eliminate discrimination against individuals and groups and to guarantee their equal right to SRH," (4, para 34). In other words, States must immediately ensure that whatever their actions, they ensure equality and non-discrimination for all, and even "implement temporary special measures to overcome long-standing discrimination... and to eradicate conditions that perpetuate discrimination," (4, para 36), where needed. Notably, the UN CESCR makes these legal obligations specific to reproductive cancers in the provision (emphasis added):

> *States should aim to ensure universal access without discrimination for all individuals, including those from disadvantaged and marginalised groups, to a full range of quality SRH care, including... [the] prevention, diagnosis, and treatment of ...* ***reproductive cancers****... (4, para 45).*

States also have 'core obligations' under the right to SRH, which signify the basic minimum level that governments must achieve in order to give meaning to the right to SRH. Among these core obligations is the duty to "guarantee universal and equitable access to affordable, acceptable and quality SRH services, goods, and facilities, in particular for disadvantaged and marginalised groups" (4, para 49c), including "access to comprehensive education and information on SRH that are non-discriminatory, unbiased, evidence-based" and that are tailored to the capacities of children and adolescents (4, para 49f), and the provision of "medicines, equipment and technologies essential to SRH, including those based on the WHO Model List of Essential Medicines" (4, para 49 g). The HPV vaccine is one of these recommended medicines, based on the 2019 WHO Model List [8]. The WHO has also included an HPV DNA testing device on its list of essential in-vitro diagnostics for healthcare facilities with clinical laboratories [9]. When read together with States' legal obligations (4, para 45), these core obligations can be understood to include non-discriminatory access to services, information, education, medicines, and technologies for the prevention and treatment of reproductive cancers.

Cervical cancer screening programmes should also be aligned with the AAAQ framework: Availability, Accessibility, Acceptability, and Quality of health goods and services necessary for the right to SRH (4, paras 11–21). Quality is of particular relevance to universal cervical cancer control. Ensuring quality services requires providing the HPV vaccine when it is most effective at preventing disease (i.e. before first sexual activity), screening for lesions as a scientific and medically appropriate measure for prevention and early detection, and providing assured quality cancer care in case of a cancer diagnosis.

Screening that reaches every woman

In most high-income countries, screening is standard practice and guidelines target women most at risk of developing cervical cancer [10, 11]. By contrast, screening is much less common in LMICs due to its high cost and the limited health infrastructure [10, 11]. A study of six LMICs found those with absent or newly implemented screening guidelines had the lowest rates of crude and effective cervical cancer screening, with high cancer incidence and mortality, while countries with established guidelines had higher screening rates and lower disease burden [10]. Even NCSPs that are explicitly designed to reach all eligible women may still miss the most disadvantaged people. NCSPs should be designed with this challenge in mind. This requires prioritising the needs of the most hard-to-reach women if these programmes truly aspire to reduce health inequalities. Evidence shows that cervical cancer-related deaths drop to ≤2 women per 100,000 when screening (with a Pap test) is done every 3–5 years and reaches 70% of eligible women [5, 12]. Achieving- and exceeding- this goal requires a move towards population-based screening instead of opportunistic screening (where the latter reaches women already in contact with the healthcare system or women presenting symptoms of cervical abnormalities). Yet, many women around the world go unscreened despite the introduction of NCSPs [13].

Indeed, social inequalities are at the heart of many screening disparities. The examples presented below illustrate commonly reported- and chronically under-addressed- screening disparities relating to: the *availability* of sufficient health facilities and human resources (Kenya); the *physical accessibility* of health services for rural and remote populations (Brazil); and the *accessibility of information* sensitive to cultural, ethnic, and linguistic barriers (Ecuador). Still, there are a number of other, often overlapping, social disadvantages that impair women's universal access to screening. Structural disadvantages that women face include difficulty registering with or navigating the health system, especially understanding one's entitlement to care, and the cost and inconvenience of travelling to or the screening services themselves, among other issues [6, 14]. A woman can face a variety of complex personal and structural barriers that exacerbate her access to screening. Therefore, in the context of cervical cancer screening, hard-to-reach women are considered to be women aged 30 to 65, who are sexually active, and who, for various reasons, are not reached by screening services and consequently, are at higher risk for cervical cancer [6, 14].

The *availability* of physicians and gynaecologists, and an "adequate number of functioning health-care facilities... to provide the population with the fullest possible range of SRH care" (4, para 12), can be a root cause of inaccessible screening. For example, the *Kenyan National Cancer Control Strategy 2017–2022* foresees piloting a population-based screening program in "counties where comprehensive regional cancer centres are being planned" [15]. Indeed, the physician-to-population ratio- a proxy measure of the availability of trained healthcare providers- varies significantly between different Kenyan counties, from 1:143,000 in a hard-to-reach community to 1:21,000 in a community with three district hospitals [16]. Although this pilot screening program is an important (first) step, a rights-based approach will plan to take "deliberate, targeted, and concrete" measures to scale-up the number of health providers reaching women beyond the areas surrounding cancer care facilities (4, para 33). Ultimately, a more comprehensive approach will be needed in Kenya, where only 3.5% of eligible women report ever being screened [13]. Consequently, in Kenya- like much of sub-Saharan Africa- cervical cancer is the leading cause of cancer-related deaths among women [17].

The example of Brazil illustrates how crucial *physically accessible* screening services "within safe physical and geographic reach for all" (4, para 16) are so that women may receive timely care, reducing the incidence of cervical cancer. Brazil employs an opportunistic screening programme that has achieved disparate levels of coverage and cancer survival across the country. Fragmented screening means that women at risk or with early-stage cancer are missed and only present to health facilities in late stages when their chances of survival are lower [18]. In particular, ensuring women can reach health facilities with trained gynaecologists and quality-controlled laboratories has limited the reach of some screening programs. Disparate access in screening services has resulted in a decrease in cervical cancer-related mortality in the developed southern, southeastern, and midwestern regions of Brazil, while an increase in the less developed areas of the northern and northeastern regions is observed [19, 20]. Consequently, the screening benefits are not enjoyed equally by all women in Brazil. Aware of these disparities, some Brazilian cancer hospitals now use mobile units, complete with laboratories, to bring screening to women in remote locations. Between 2002 and 2012, these vehicles navigated difficult terrain without roads, animal herds, and water crossings via ferry to screen 174,605 women who were unlikely to have otherwise been tested for cervical cancer or its precursors [21].

Information and education about cervical cancer screening, diagnosis, and preventative treatment should be tailored and *accessible* to hard-to-reach women. These measures are consistent with the 'core obligations' of comprehensive education and information about how to prevent, diagnose and treat sexually-transmitted infections, such as HPV, and reproductive cancers (4, para 49f). For example, despite providing free-of-charge cervical cancer screening at health facilities, Ecuador has low national screening coverage (9–23% of eligible women) and a high incidence of cervical cancer (19 women per 100,000) [22, 23]. In order to improve this picture, attention should be paid to indigenous people's needs as they constitute 1.1 million of the 16.4 million people in Ecuador, yet are often marginalised in health matters [24]. Indigenous women face multiple forms of discrimination when accessing screening provided in health centres: language is a barrier for non-Spanish speakers to access care and these women may experience judgement by healthcare providers [25, 26]. Individual barriers are also at play, such as gender norms, cultural customs, and a mistrust of Western medicine (that are possibly related to past mistreatment), which inhibit these women from undergoing a Pap test [26, 27]. Although these factors can have a chilling effect on women's screening attendance, it is most telling that information promoting screening has failed to reach indigenous women. Some indigenous women report first learning about the Pap test when receiving primary care for their first pregnancy or after their first child [27]. A holistic public health and human rights approach requires that information about sexually-transmitted infections and reproductive cancer be *accessible* and "provided in a manner consistent with the needs of the

individual and the community, taking into consideration, for example, age, gender, language ability, education level, disability, sexual orientation, gender identity, and intersex status" (4, para 18–19).

Vaccination and screening

HPV vaccination is a promising strategy to prevent high-risk infections. The HPV vaccines are extremely effective at preventing infections by common high-risk HPV types [28, 29]. Yet HPV vaccination is most effective when given before women are exposed to the virus. This limitation means that women who are vaccinated before their first sexual activity will benefit most from HPV vaccination, not women who are already sexually active [5]. Eradicating HPV infections through vaccination is currently difficult because most immunisation programmes only target girls and women, while boys and men can also transmit the virus and develop HPV-related cancers [5]. Herd immunity will be more easily reached when all potential carriers are vaccinated. Based on the latest demography updates, HPV vaccination provides more health benefits and is more cost-effective than previously estimated [30].

The widescale implementation of the nonavalent vaccine – US Food and Drug Administration (FDA) approved in 2014 - will be another crucial step towards stopping HPV transmission. The vaccine offers protection to 9 genotypes, of which 7 oncogenic (versus 2 high-risk HPV types in the previously approved vaccines), and is clinically proven to prevent HPV-related diseases in both sexes [31].

However, HPV vaccines can only achieve these public health gains if they are available and affordable for women and health systems [4]. Many countries, some with a high burden of cervical cancer, experience significant lag time in implementing the HPV vaccine in national programmes despite its approval by the FDA over 10 years ago [32]. It is also important to remember that HPV vaccines cannot treat pre-existing HPV infections nor cervical cancer itself. For these reasons, the public health benefits of recent HPV vaccinations will only be evident in several decades [5]. In addition, vaccination alone is unlikely to lead to cervical cancer eradication. Therefore, vaccination should be complemented with screening to detect treatable pre- or early-stage cancer before it enters advanced stages [5].

Traditionally, cervical cancer screening is done by cytology, where a physician, gynaecologist, or other trained sampler (i.e. nurse or midwife) collects a sample of cervical cells (commonly called a Pap test) and evaluates it for the presence of cell abnormalities under a microscope. This method was introduced in 1941 and is credited with achieving a 70% reduction in cervical cancer rates in the USA [33]. However, processing cytological tests is highly dependent on a sufficient number of trained health providers to collect samples, and having access to sophisticated laboratory equipment and highly qualified pathologists to interpret the results. A number of quality concerns can be triggered when laboratories process either too few tests annually to maintain their skills or overload technicians with too many tests, both risking diagnostic errors [20]. Moreover, interpreting the test is time consuming and inherently subjective, with limited reproducibility and sensitivity to detect pre-cancer [34]. Some women might also require a second consultation if test results are atypical, undefined, to conduct further testing or begin treatment.

In places unable to support high-quality cytology, visual inspection of the cervix with acetic acid, by a trained health provider, is a low-cost and simple alternative often recommended [5]. Indeed, alternative approaches to cervical screening in resource-constrained settings have been adopted including screening women once in a lifetime using visual inspection with acetic acid (VIA) or HPV testing, which has been found to reduce lifetime risk of cancer by approximately 30% and cost less than US$500 per year of life saved [35]. Visual inspection-based screening looks for a colour change of the cervix when acetic acid is applied. While the sensitivity of VIA can be improved, the low specificity also leads to many false positives and over treatment [36]. Therefore, ensuring access to quality services remains a major barrier to scaling-up universal screening programmes based on cytology or visual inspection in many LMICs.

Now, new technologies to detect HPV DNA offer a number of advantages over cytology, which can reduce disparate access to cervical cancer screening. An HPV test uses a sample of cells from a woman's vagina/cervix to detect the presence of high-risk subtypes of HPV DNA that increase her risk of cervical cancer. It is equally effective and a more sensitive strategy than cytological evaluation alone [37, 38]. The WHO's 2013 Guidelines for screening and treatment of cervical cancer endorse HPV tests if the programme has sufficient resources, promoting cytology only if it meets quality indicators [39]. The 2015 European Guidelines for Quality Assurance in Cervical Cancer Screening recommend the implementation of HPV tests as a primary screening strategy [37]. Although most HPV DNA tests still require laboratory infrastructure, some new devices make it possible to bring the lab to the patient in a single, handheld test (discussed further below) [40].

Modern screening methods to overcome disparities

NCSPs offering screening services and evidence-based technologies aligned with human rights, technological advances and modern clinical practice, and fit with the needs of both the general population and hard-to-reach

populations of women, have the highest potential to achieve more [18]. Having multiple screening methods and follow-up strategies within a NCSP to increase coverage and continuity of care, respectively, is therefore an alternative that more programmes should consider. Cytology is still the most common national cervical cancer screening method, despite the difficulty implementing them in low-resource settings (i.e. without trained gynaecologists and pathologists, and laboratory facilities) [41]. In this regard, HPV DNA testing offers several advantages over cytology for expanding screening with limited resources to hard-to-reach populations.

First, HPV DNA testing allows women to take self-samples (i.e. to collect cells from herself using a vaginal swab), which is not compatible with cytology. Self-sampling is associated with higher screening coverage, particularly among vulnerable populations facing linguistic, cultural, geographic, or economic barriers [42–44]. It is also highly accepted by women because it is more discrete and less invasive than physician-obtained samples [45]. Furthermore, self-sampling "has the potential to further empower women to collect their own samples in privacy giving them control over how and when they participate in screening" [46]. HPV test results obtained in self-sampled material are highly concordant with those obtained by physicians [47–51]. Self-sampling is a strategy to increase screening participation, particularly among hard-to-reach women, because it can be safely and effectively done with support from community health workers (instead of physicians or gynaecologists) or by women alone [48, 52].

Second, screening by self-sampling in populations of hard-to-reach women can be further enhanced if used together with a user-friendly HPV rapid testing device. One of the remaining limitations of HPV testing is its reliance on laboratory infrastructure to process the results. However, HPV rapid testing devices can alleviate this constraint by bringing a portable molecular DNA test to women. Some of the present authors are part of the international research consortium, ELEVATE, which recently launched a five-year project financed by the European Commission's Horizon 2020 programme to develop a new test and screening approach for cervical cancer in hard-to-reach women. The test will combine self-sampling with a new low-cost, portable measurement device that will be validated in screening trials in Belgium, Brazil, Ecuador, and Portugal. The new ELEV ATE HPV test will yield easy-to-understand results in low-resource settings lacking specialist health personnel or electricity in remote locations. Point-of-care results mean that women can be screened and receive their results in the same visit, resulting in increased continuity of care and efficient follow-up processes. How such a device can be an added value to under-screened women is highly context dependent (i.e. home-based self-sampling, community mobilisation for testing, testing followed by clinician counselling, or in other combinations) [53]. Therefore, the ELEVATE project will also execute pilot studies in hard-to-reach populations to determine the feasibility, user and health provider acceptability, costs, logistics, and population compliance of self-sampling and the rapid HPV testing device [54, 55].

The budget impact of the HPV test has been a barrier to its widespread introduction in some LMICs. Nevertheless, NCSPs should consider a self-sampling and HPV testing method to reach under-screened women, remembering that governments may need to devote "greater resources to [these] traditionally neglected groups" to eliminate systemic discrimination and ensure their right to SRH (4, para 31). The cost-effectiveness of HPV testing methods has been shown in various contexts, while maintaining or even improving effectiveness compared to traditional cytology programmes [56]. Studies on primary HPV testing, including self-sampling methods, followed by triage for HPV-positive cases are also shown to be cost-efficient and effective in Brazil [57]. In Canada, for example, self-testing in rural populations, when combined with community engagement and education, is effective at increasing coverage in underserved populations and is a cost effective alternative [52].

Conclusion

The crux of cervical cancer control rests in prevention through vaccination and early detection through screening. Until now, many NCSPs have been unresponsive to important social inequalities that marginalise some groups of women, hampering universal access to screening services. Under-screened women have a higher burden of cervical cancer and worse survival rates than regularly-screened women. Yet, there is good reason for Dr. Tedros to call cervical cancer "a NCD we can overcome" [2]. Governments have human rights obligations to refocus screening policies and programmes on women who are disproportionately affected by discrimination that impairs their full enjoyment of the right to SRH (4, paras 30–31). To reach underserved women, NCSPs also rely on and can contribute to strengthening the six building blocks of health systems: (1) innovative approaches to health services, such as screening through mobile health units and community health workers; (2) health information systems to manage immunisation and screening records, and community outreach in a language and manner that is *acceptable* to the target population; (3) an adequate number of trained health-care providers to immunise, screen/sample, and interpret test results; (4) regular supply of self- sampling and point-of-care HPV testing devices that are *acceptable* to marginalised women and health providers, and of HPV

vaccines (from a temperature-assured supply chain) that are *available* at an *affordable* price; (5) adequate financing for the foregoing measures; and (6) leadership and good governance to implement these measures through out the health system with particular attention for hard-to-reach populations [58]. NCSPs should tailor screening methods to under-screened women, bearing in mind that eliminating systemic discrimination may require "devoting greater resources to traditionally neglected groups" (4, para 31). NCSPs that keep these human rights principles central will be able to expand screening among low-income, isolated and other marginalised populations, but also women in general, who, for a variety of reasons, do not visit healthcare providers for regular screenings. With so much political will and global momentum towards eliminating cervical cancer as part of NCD control, the time is right to invest in evidence-driven, rights-based, innovative screening practices that target underserved women globally.

Abbreviations

FDA: US Food and Drug Administration; HPV: Human papillomavirus; ICESCR: International Covenant on Economic, Social, and Cultural Rights; LMICs: Low- and middle-income countries; NCD: Non-communicable diseases; NCSP: national cervical cancer screening programmes; SRH: Sexual and reproductive health; UN CESCR: United National Committee on Economic, Social, and Cultural Rights; WHO: World Health Organization

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Authors' contributions

KP conceptualized the manuscript and completed the first draft with HV and AW. KP, HV, AW, SB-A, EDP, SD, AG, IK, AL-F, JO, EP, RMR, NV, BV, BV, OD assisted with information acquisition and interpretation; revised the manuscript; approved its final content; and agree to be personally accountable for their contributions.

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