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POP-Brazil Study Protocol: a nationwide cross-sectional evaluation of prevalence and genotype distribution of human papillomavirus (HPV) in Brazil

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Keywords:	HPV prevalence, nationwide study, HPV infection, cervical, penile

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Manuscripts

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3 **POP-Brazil Study Protocol: A nationwide cross-sectional evaluation of the prevalence and**
4 **genotype distribution of human papillomavirus (HPV) in Brazil**
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ABSTRACT

Introduction: Human papillomavirus (HPV) is associated with the development of genital warts and different types of cancer, including virtually all cervical cancers and a considerable number of penile, anal and oropharyngeal cancers. Data regarding the prevalence of HPV infection in Brazil are restricted and fragmented. We aim to determine the HPV prevalence in sexually active women and men from 16 to 25 years old and to investigate regional differences in virus prevalence and types. **Methods and analysis:** This is a nationwide, multi-centric, cross-sectional, prospective study that will include participants from 16 to 25 years old from all Brazilian capital cities. Recruitment will occur in primary health units by trained health professionals who will be responsible for collecting biological samples and interviewing the volunteers. After signing an informed consent form, all participants will answer a questionnaire that will gather sociodemographic and behavioral data. All samples will be processed in a certified central laboratory, and strict quality control will be performed by many different procedures, including double data entry, the training and certification of primary care health professionals responsible for data collection, the simulation of interviews and the auditing and monitoring of visits. The sample size will be standardized by the population distribution of each capital, using SAS[®] and R statistical software. **Ethics and dissemination:** The project was submitted to the Research Ethical Committee of the main institution and the corresponding ethical committees of the 27 recruiting sites. This will be the first Brazilian nationwide study to determine the overall HPV prevalence and to look at regional differences and social, demographic and behavioral factors related to HPV infection. Critical analysis of the study results will contribute to epidemiological knowledge and will set a baseline to evaluate the impact of the National HPV Vaccination Program in the future.

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5 **Keywords:** HPV prevalence, nationwide study, HPV infection, cervical, penile
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10 **Strengths and limitations of this study**
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- 12 • Representativeness of all regions of Brazil.
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14 • Establishment of a baseline to evaluate the effectiveness of the HPV vaccination program
15 in Brazil.
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17 • Recruitment done in primary care units, inviting all people within the age range living in
18 the area and not over representing risk factor groups.
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20 • Only the population that uses the primary care units from the public health system will be
21 included, but that consists of 71% of the Brazilian population.
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INTRODUCTION

According to the World Health Organization (WHO), more than 528,000 incident cases and approximately 270,000 deaths due to cervical cancer occur annually worldwide, 85% of which occur in low- and middle-income countries.[1] Virtually all these cancers are preceded by infection with high-risk oncogenic human papillomaviruses (HR-HPVs),[2] and HPV types 16 and 18 are associated with 70% of the cases.[3] In Brazil, the National Institute of Cancer estimated that there were 16,340 new cervical carcinoma cases in 2016, with a 33.23% mortality rate.[4]

HR-HPVs are also responsible for 63% of penile cancers, 89% of anal cancers and 72% of oropharyngeal cancers in men.[5, 6] Indeed, these viruses are now recognized as the major cause of the observed increasing incidence rates of oropharyngeal squamous cell carcinoma, and the primary risk factor for developing this cancer is oral HPV infection.[7]

Most sexually active individuals will be infected by HPV at some point in their lives.[8, 9] The prevalence of HPV infection in women with normal cervical cytology in Brazil varies from 2.3% to 55.4%.[10, 11] Other body sites, such as the penile epithelium and the oral cavity, also present considerable variability in HPV prevalence rates in Brazil.[10, 11]

These data came from heterogeneous studies regarding their methodology and/or the study population. In addition, virtually all studies are geographically restricted[12-16] and/or focused on specific groups of individuals, such as HIV-positive, pregnant and immunosuppressed patients.[17-19] Moreover, some of those studies recruit participants from clinics specializing in sexually transmitted infections, causing a selection bias and inflating the prevalence estimation.[12-19]

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3 Vaccination against HPV is being performed in many countries, and it is a method of
4 primary prevention of cancer and HPV infection.[8] Since 2014, the Brazilian Ministry of Health
5 has provided a quadrivalent (types 6, 11, 16 and 18) vaccine for women from 9-14 years old,
6 with a schedule of 0 and 6 months. In January 2017, Brazil was the first country in Latin
7 America to extend vaccination to men (11-14 years old).[20] Baseline data of HPV prevalence
8 before vaccinations are vital in order to establish the impact of vaccination on the distribution of
9 HPV types.
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19 As far as we know, this is the first study presenting a nationwide scope with uniform
20 methodology to evaluate the Brazilian prevalence of HPV and its types, as well as to compare
21 the prevalence among specific geographical regions that present marked differences in social,
22 demographic, cultural and economic characteristics. In addition, the study will describe the
23 social, demographic, economic and behavioral factors associated with HPV positivity.
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33 **OBJECTIVES**

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35 The primary objective of the study is to determine the prevalence of HPV in women and
36 men aged 16-25 in Brazil, evaluating the most prevalent types and possible differences between
37 regions and risk factors associated with positivity.
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45 **Secondary objectives**

- 46 • To determine the prevalence of HPV, high-risk types, genital warts and the
47 associated sexual behaviors;
- 48 • To evaluate the co-infection of HPV-HIV in the young population that uses the
49 public health system;
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- To investigate beliefs and knowledge about HPV infection and vaccination in a young population;
- To evaluate the association of other sexually transmitted infections (STI), such as syphilis, with the presence of HPV;
- To establish an HPV prevalence baseline to evaluate vaccine effectiveness.

METHODS AND ANALYSIS

Design

We will use a cross-sectional, nationwide, multi-centric design to establish a baseline dataset that will enable prevalence comparison over time.

Recruitment and sample size

Sexually active women and men from 16 to 25 years old who use the public health system in all 26 Brazilian capitals plus the Federal District of Brasilia across the five geographical regions will be recruited through public primary care units.

The following exclusion criteria apply: pregnant women, those who delivered a baby in the last 3 months, those who have undergone a hysterectomy or trachelectomy and those who have ever had cervical intraepithelial neoplasia grade 2 or higher will be considered ineligible for the study.

There will be no exclusion based on HPV vaccination status, but we will investigate whether the participants have been vaccinated. Since the vaccination program was launched in

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3 2014 for women who were 9-13 years old and our study population is among 16-25-year-olds,
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5 we believe that most of the volunteers will be not have been vaccinated.
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8 The units were selected based on their representativeness of the health districts and based
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10 on their resources to collect and store oral samples. Most of these units have a defined territory
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12 and a list of all people within the age range. When the list is available, all participants will be
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14 invited. When the list is not available, participants will be recruited by different approaches: (i)
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16 eligible individuals who came to the unit for any reason not associated with the main study's
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18 outcome; (ii) personal invitation by the community health agents; and (iii) invitation by nurses in
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20 the school-based health promotion program. The study sample size (7,935 individuals) was
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22 calculated to detect differences of 5% in HPV prevalence between regions with a power of 80%
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24 and an alpha error of 5% for women, using an estimate of 30%[21] for the prevalence of HPV in
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26 Brazil. This sample size will allow an estimate with an error between 1.6% and 5.7%, according
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28 to sex.
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33 The sample size will be purposely equal in all regions (1,587 individuals) to maximize
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35 the diversity in less populated areas and will be standardized by the Brazilian population of each
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37 region during analyses.
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42 **Measurements**

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47 Sociodemographic variables

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49 Participants will be asked to answer questions regarding age, gender, race/skin color,
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51 household characteristics, relationship status, educational level, occupational status, last-month
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53 family income and the number of people dependent on this income.
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Smoking, alcohol and other drugs

Questions concerning current and past smoking habits, including quantity and frequency, will be asked of the participants. Moreover, the interview includes questions about the use of alcohol and other drugs such as marijuana, cocaine, ecstasy, crack, and the abuse of morphine or other licit and illicit drugs.

Sexual and reproductive health

Participants will be asked to supply details about their age at first sexual intercourse, their involvement in same sex relationships, their use of condoms, the number of sexual partners, their sexual practices and the presence of any genital symptoms during or after sexual intercourse such as pain or bleeding.

Their age of menarche, the number of pregnancies and deliveries, the number of abortions, their use of contraceptive methods and the occurrence of sexually transmitted infections will be asked of participants to evaluate their sexual health. All suspicious HPV-related lesions will be photographed for future evaluation.

Knowledge about HPV and vaccination

We will measure how much participants know about HPV and vaccination (when and who must receive the vaccine). The questionnaire is based on Saulle et al.,[22] Moreover, participants will be asked if they know about Pap smears, if they have already received the exam and if so, if any alteration was ever observed.

HPV frequency and type

We will measure the proportion of the sample that is infected by HPV, and we will look at the prevalence of high-risk types and the most prevalent types in the genital and oropharyngeal sites. Additionally, we will investigate the presence of chronic lesions (lasting 15 days or more) in the oral cavity that are related to the presence of HPV.

Outcomes

The nationwide prevalence of HPV in the genitals is the major outcome. Secondary outcomes include the prevalence of specific HPV types, the comparison of prevalence between Brazilian regions, and the prevalence of HR-HPVs and LR-HPVs.

Procedures

All individuals will respond to a standardized questionnaire based on validated instruments[22-25] and will undergo the collection of specimens from the oral cavity and the cervical or penile/scrotal sites. Oral samples will be obtained through mouthwash and gargle.

An online platform for data entry will be used by the study staff to include participant data, biological sample information and photographs. The same platform will be used for study process control and to make the results available to health professionals and participants.

Cervical samples will be obtained using the Digene® HC2 DNA Collection Device (Qiagen), and penis (penile shaft, glans penis/coronal sulcus) and scrotum samples will be obtained using a Dacron swab (Qiagen) previously humidified in sterile saline solution by intensely pressing and rubbing the swab in the epithelium.

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3 Individuals who present visible lesions on the genitals or in the oral cavity at the time of
4 recruitment will be asked to grant permission for a picture to be taken of the lesion.
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8 All biological samples will be maintained at room temperature (15°C to 25°C) according
9
10 to manufacturer's instructions and transported to a central lab in Porto Alegre to start the
11 specimen processing. The temperature will be monitored with a data logger. Figure 1 presents
12 the general logistics for the nationwide multicenter collection of data and specimens.
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16 17 18 19 Specimen processing 20

21 In the laboratory, specimens will be immediately aliquoted. One aliquot will be
22 maintained at -80°C as a backup. Another aliquot will be processed for DNA extraction using the
23 automatized extraction platform MagNA Pure[®] (Roche), following the manufacturer's
24 instruction.
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31 HPV detection and typing will be done using the Linear Array[®] HPV Genotyping Test
32 (Roche Diagnostics), which is based on PCR amplification (450-bp fragment of the polymorphic
33 region of the L1 gene of HPV), followed by specific hybridization and colorimetric detection.
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35 The test detects HR-HPVs (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and LR-HPVs
36 (6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39 and
37 CP6198), for a total of 37 types. The amplification of human β -globin (biotinylated
38 primers GH20 and PC04, 268-bp fragment) is used as an internal control for the PCR. Whenever
39 necessary, real-time PCR using the TaqMan system will be performed to confirm the HPV type.
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Data analyses and statistics

Categorical variables will be summarized using absolute frequencies and percentage, while continuous variables will be analyzed using means and standard error. Chi-squared tests and Fisher's exact tests will be used to compare proportions, and t-tests or Mann-Whitney non-parametric tests will be used for continuous variables.

The association between HPV infection and social and behavioral variables will be defined by calculating crude and adjusted prevalence ratios. Confounding variables will be evaluated using a modified Poisson regression analysis.[26]

To maximize diversity across regions, we have divided the sample size into equal numbers among regions. To adjust the distribution of the sample to the study population, we will standardize the measures by the size of the population in each capital within the age range and by sex.

Monitoring and quality control

The data quality will be validated by routine practices. A pilot study was performed to identify and correct problems in data collection, sample transportation, procedures and instruments. All health professionals involved in the research are trained and certified by the coordinator team using simulated interview and sample collection according to the study protocol, as described in the POP-Brazil operations manuals.

Data and specimen collection are periodically evaluated by reports generated by the data system, including information about the number of participants and the number of refusals, and the quality of the samples collected. Those reports will be distributed monthly to all people involved in data collection.

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3 Moreover, each unit will receive at least one *in loco* monitoring visit, which will be done
4 by research staff supervisors to evaluate protocol compliance and provide recertification.
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6 Supervisors will observe techniques utilizing predefined check lists and recorded interviews.
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10 The biological sample tracking is done through the web-based data platform, and time,
11 temperature and sample conditions are evaluated on a daily basis.
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14 To access the reliability of the interviews, a subsample will be obtained in duplicate. A
15 resampling will be done in 10% of the interviews using phone calls to access the coefficient of
16 variation and systematic differences between data. Figure 2 presents the steps that will be taken
17 for quality assurance. The reliability of HPV detection will be accessed by double processing a
18 random subsample of the biological genital samples by the Centers for Disease Control and
19 Prevention.
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33 **DISCUSSION**

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35 This is the first Brazilian nationwide study to determine HPV prevalence and the social,
36 demographic and behavioral factors related to HPV infection. Critical analysis of the study
37 results will contribute to epidemiological knowledge of the distribution of HPV strains across
38 geographical regions and will establish a baseline to evaluate the impact of the Brazilian
39 Vaccination Program.
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47 There are some limitations of this study. The sampling is not random. Brazil is a large
48 country, and due to logistic reasons, the sampling is restricted to state capitals in public health
49 units selected by the local government. To minimize selection bias, in addition to recruiting
50 participants that voluntarily come to the health unit, we will also recruit at schools, through the
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3 list of patients of the selected health units, and by personal invitation by community health
4 agents in households. We are also only recruiting participants in the public health system, which
5 account for approximately 70% of the population, and therefore, we cannot make inferences
6 about people who exclusively use the private sector.
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12 The strengths of the study are the establishment of a baseline for future comparison of
13 HPV prevalence, the nationwide sampling, including a large and diverse population and the use
14 of central laboratory and techniques for data quality monitoring. Additionally, we will collect
15 data on sexual behaviors and other STIs, making it possible to pursue some explanation
16 regarding differences in prevalence according to specific groups of participants.
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24 Health managers, based on these data, will be able to compare HPV prevalence in
25 different regions according to population characteristics; it will be possible to support the
26 development of strategies to promote HPV vaccination and to develop strategies for prevention,
27 taking into account regional differences. Moreover, these data will serve as a baseline for future
28 comparisons and will allow monitoring of the effectiveness of the National HPV Vaccination
29 Program.
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40 ETHICS AND DISSEMINATION

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42 Before starting the study, this project was submitted to the Research Ethical Committee
43 of the proposing institution (Hospital Moinhos de Vento). Following approval, agreements were
44 made by all co-participant centers and submitted to their corresponding ethical committees.
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49 Individuals must voluntarily agree to participate and must sign the free and informed
50 consent form. The study follows the ethical recommendations of Resolutions 347/05, 441/11 and
51 466/12 of the National Health Council.
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3 Patients will be informed of negative results via web access, and patients with positive
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5 results will be advised to go to the health care unit for their results, where they will be educated
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8 about healthy sexual habits and STI prevention.
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AUTHOR'S CONTRIBUTION

EMW conceived of the project and participated in all phases of the manuscript preparation; ASB, AGM, LSH, MFAS, JH, FM, LLV, CMD and MB participated in the protocol development; and JC and LLV participated in the laboratory protocol development and helped write the manuscript. All authors reviewed the final version of the manuscript.

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COMPETING INTERESTS

ASB, FMAS, CMD and AGM work for the Ministry of Health of Brazil. The other authors declare no conflict of interest. LLV is a consultant for Merck for the HPV quadrivalent vaccine and for Qiagen, BD and Roche for HPV DNA tests.

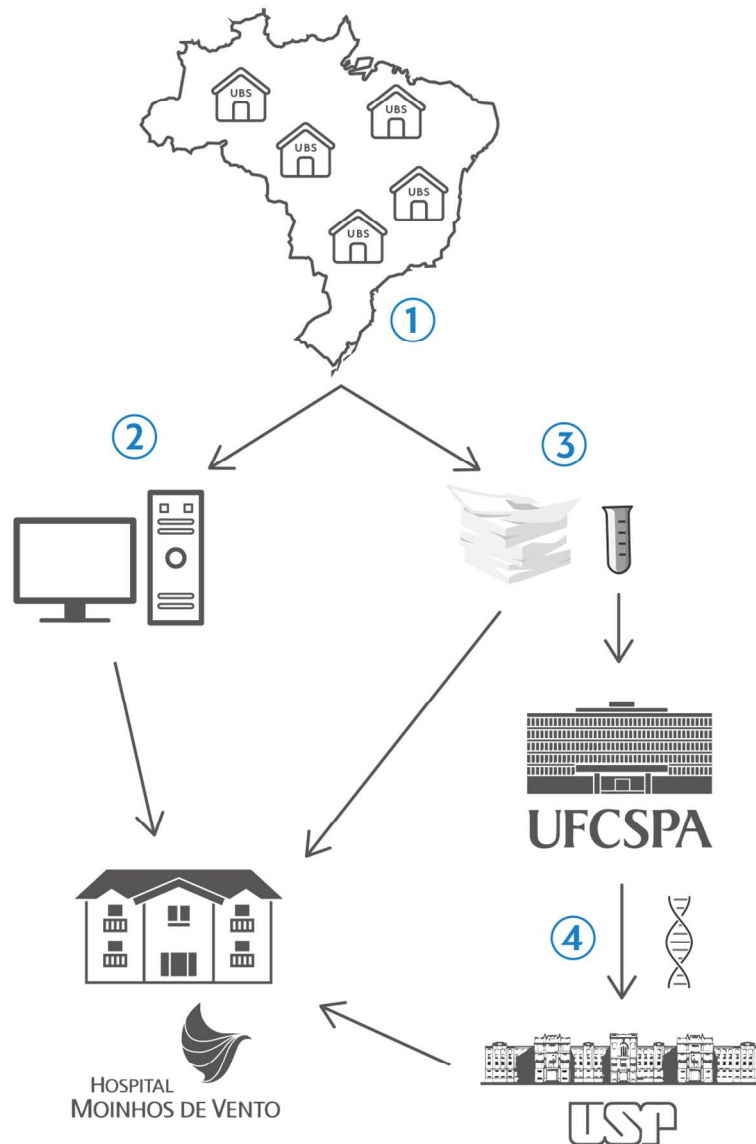
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Figure legends

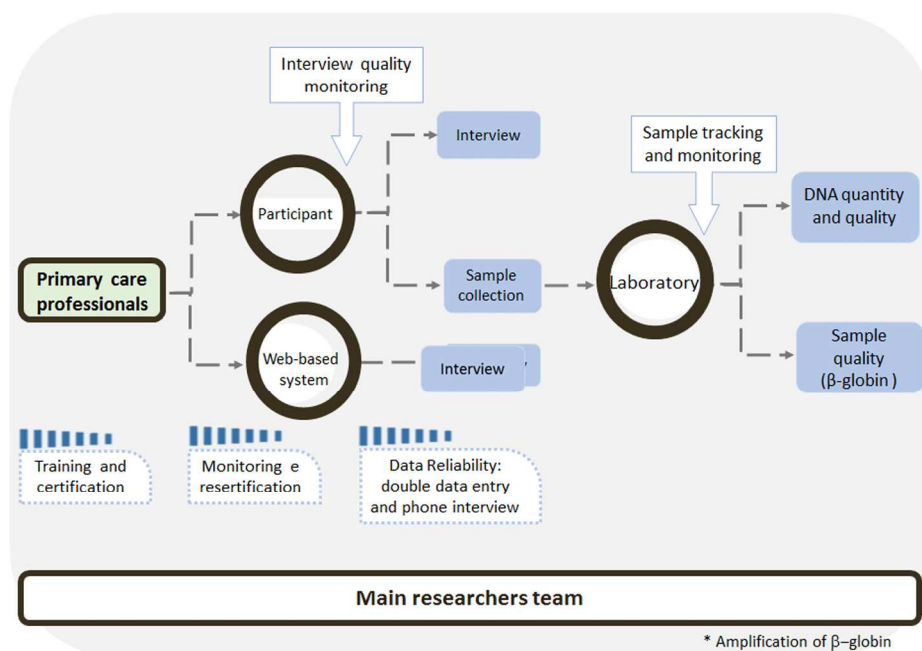
Figure 1. Workflow of the study. 1. Data are collected in public health units from all 27 Brazilian capitals; 2. Data entry into the web-based system is performed by primary care professionals and is monitored by the main research team in Porto Alegre (Hospital Moinhos de Vento); 3. Paper forms are sent to Porto Alegre along with biological samples, which are processed for DNA extraction (UFCSPA); and 4. Extracted DNA is sent to São Paulo (USP) for HPV detection and typing. Finally, the results are included in the web-based system and are made available for the primary care professionals and participants.

Figure 2. Description of the study's major quality control steps during each phase of the study.



Workflow of the study. 1. Data are collected in Public Health Units from all 27 Brazilian capitals; 2. Data entry on the web-based system are performed by primary care professionals and are monitored by main research team in Porto Alegre (Hospital Moinhos de Vento); 3. Paper forms are sent to Porto Alegre along with biological samples, which are processed for DNA extraction (UFCSPA); 4. Extracted DNA are sent to São Paulo (USP) for HPV detection and typing. Finally, results are included on the web-based system and became available for primary care professionals and participants.

106x143mm (300 x 300 DPI)



Description of the study quality control major steps during each phase of the study.

297x209mm (300 x 300 DPI)

BMJ Open

POP-Brazil Study Protocol: a nationwide cross-sectional evaluation of the prevalence and genotype distribution of human papillomavirus (HPV) in Brazil

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Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Sexual health, Public health, Infectious diseases
Keywords:	nationwide study, HPV infection, anogenital, prevalence, Brazil, EPIDEMOLOGY

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Manuscripts

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3 1 **POP-Brazil Study Protocol: A nationwide cross-sectional evaluation of the prevalence and**
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5 2 **genotype distribution of human papillomavirus (HPV) in Brazil**
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For peer review only

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3 **30 ABSTRACT**
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5 **31 Introduction:** Human papillomavirus (HPV) is associated with the development of genital warts
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8 **32** and different types of cancer, including virtually all cervical cancers and a considerable number
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10 **33** of penile, anal and oropharyngeal cancers. Data regarding the prevalence of HPV infection in
11
12 **34** Brazil are limited and fragmented. We aim to determine HPV prevalence in sexually active
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14 **35** women and men from 16 to 25 years old and to investigate regional differences in virus
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16 **36** prevalence and types. **Methods and analysis:** This is a nationwide, multi-centric, cross-
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18 **37** sectional, prospective study that will include participants from 16 to 25 years old from all
19
20 **38** Brazilian capital cities. Recruitment will occur in primary health units by trained health
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22 **39** professionals who will be responsible for collecting biological samples and interviewing the
23
24 **40** volunteers. After signing informed consent, all participants will answer a questionnaire that will
25
26 **41** collect sociodemographic and behavioral data. All samples will be processed in a certified
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28 **42** central laboratory, and strict quality control will be performed by many different procedures,
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30 **43** including double data entry, training and certification of primary care health professionals
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32 **44** responsible for data collection, simulation of interviews and auditing and monitoring of visits.
33
34 **45** The sample size will be standardized based on the population distribution of each capital using
35
36 **46** SAS[®] and R statistical software. **Ethics and dissemination:** The project was approved by the
37
38 **47** Research Ethics Committee of the main institution and the corresponding ethics committees of
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40 **48** the recruitment sites. This will be the first Brazilian nationwide study to determine overall HPV
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42 **49** prevalence and to examine regional differences and social, demographic and behavioral factors
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44 **50** related to HPV infection. Critical analysis of the study results will contribute to epidemiological
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46 **51** knowledge and will set a baseline for future evaluation of the impact of the National HPV
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48 **52** Vaccination Program.
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45 54 **Keywords:** HPV infection, prevalence, nationwide study, Brazil, anogenital
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910 56 **Strengths and limitations of this study**
1112 57 • Representativeness of all regions of Brazil.
1314 58 • Establishment of a baseline to evaluate the effectiveness of the HPV vaccination program
15 in Brazil.
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17 5918
19 60 • Recruitment done in primary care units, inviting all people within the age range living in
20 the area and not over representing risk factor groups.
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22 6123
24 62 • Only the population that uses the primary care units from the public health system will be
25 included, but that consists of 71% of the Brazilian population.
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65 INTRODUCTION

66 According to the World Health Organization (WHO), more than 528,000 incident cases
67 and approximately 270,000 deaths due to cervical cancer occur annually worldwide, 85% of
68 which occur in low- and middle-income countries [1]. Virtually all these cancers are preceded by
69 infection with high-risk oncogenic human papillomaviruses (HR-HPVs) [2], and HPV types 16
70 and 18 are associated with 70% of these cases [3,4] In Brazil, the National Institute of Cancer
71 estimated that there were 16,340 new cervical carcinoma cases in 2016, with a 33.23% mortality
72 rate [5].

73 HR-HPVs are also responsible for 63% of penile cancers, 89% of anal cancers and 72%
74 of oropharyngeal cancers in men [6,7]. Indeed, these viruses are now recognized as the major
75 cause of the observed rising incidence rates of oropharyngeal squamous cell carcinoma; indeed,
76 the primary risk factor for developing this cancer is oral HPV infection [8].

77 Most sexually active individuals will be infected by HPV at some point in their lives
78 [9,10]. In Brazil, the prevalence of HPV infection in women with normal cervical cytology
79 varies from 2.3% to 55.4% [11,12]. Considerable variability in HPV prevalence rates for other
80 body sites, such as the penile epithelium and the oral cavity, are also observed in Brazil [11,12].

81 These data originated from studies that were heterogeneous with regard to methodology
82 and/or the study population. In addition, virtually all studies to date were geographically
83 restricted [13–17] and/or focused on specific groups of individuals, such as HIV-positive,
84 pregnant and immunosuppressed patients [18–20]. Moreover, some of those studies recruited
85 participants from clinics specializing in sexually transmitted infections, resulting in selection bias
86 and inflation of the estimated prevalence [13–20].

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3 87 Vaccination against HPV is being performed in many countries, and it is a method of
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5 88 primary prevention of cancer and HPV infection [9]. Since 2014, the Brazilian Ministry of
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7 89 Health has provided a quadrivalent (types 6, 11, 16 and 18) vaccine for women from 9-14 years
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9 90 old, with a schedule of 0 and 6 months. In January 2017, Brazil was the first country in Latin
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11 91 America to extend vaccination to men (11-14 years old) [21]. Baseline data of HPV prevalence
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13 92 before vaccinations are vital in order to establish the impact of vaccination on the distribution of
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15 93 HPV types.

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19 94 As far as we know, this is the first study presenting a nationwide scope with uniform
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21 95 methodology to evaluate the Brazilian prevalence of HPV and its types, as well as to compare
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23 96 the prevalence among specific geographical regions that present marked differences in social,
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25 97 demographic, cultural and economic characteristics. In addition, the study will describe the
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27 98 social, demographic, economic and behavioral factors associated with HPV positivity.
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32 33 100 **OBJECTIVES**

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35 101 The primary objective of the study is to determine the prevalence of HPV in women and
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37 102 men aged 16-25 years in Brazil, evaluating the most prevalent types and possible differences
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39 103 between regions and risk factors associated with positivity to establish a baseline for evaluation
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41 104 of vaccine effectiveness.
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45 46 47 106 **Secondary objectives**

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49 107 • To determine the prevalence of HPV, high-risk types, genital warts and the
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51 108 associated sexual behaviors;
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3 109 • To evaluate the co-infection of HPV-HIV in the young population that uses the
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5 110 public health system;
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8 111 • To investigate beliefs and knowledge about HPV infection and vaccination in a
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10 112 young population;
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12 113 • To evaluate the association of other sexually transmitted infections (STIs),
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14 114 such as syphilis, with the presence of HPV;
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17 115 • To evaluate the herd effect in a sample of unvaccinated men.
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21 22 117 **METHODS AND ANALYSIS**

23 24 118 25 26 119 **Design**

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29 120 We will use a cross-sectional, nationwide, multi-centric design to establish a baseline
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31 121 dataset that will enable prevalence comparison over time.
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33 122 34 35 123 **Recruitment and sample size**

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38 124 Beginning in January 2017, sexually active women and men from 16 to 25 years old who
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40 125 use the public health system in all 26 Brazilian capitals plus the Federal District of Brasilia
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42 126 across the five geographical regions will be recruited through public primary care units

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45 127 Because endocervical collection is not recommended during pregnancy in Brazil and/or
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47 128 to avoid selection bias, the following exclusion criteria will be applied: pregnant women, those
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49 129 who delivered a baby in the last 3 months, those who have undergone a hysterectomy or
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51 130 trachelectomy and those who have ever had cervical intraepithelial neoplasia grade 2 or higher
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54 131 will be considered ineligible for the study.
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3 132 There will be no exclusion based on HPV vaccination status, but we will investigate
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5 133 whether the participants have been vaccinated. Since the vaccination program was launched in
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7 134 2014 for women who were 9-13 years old and our study population is among 16-25-year-olds,
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10 135 we believe that most of the volunteers will be not have been vaccinated.

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12 136 The primary care units in each city were selected based on their representativeness of the
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14 137 health districts and based on their resources to collect and store oral samples. Most of these units
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16 138 have a defined territory and a list of all people within the age range. When the list is available, all
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18 139 participants will be invited. When the list is not available, participants will be recruited by
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20 140 different approaches: (i) eligible individuals who came to the unit for any reason not associated
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22 141 with the main study's outcome; (ii) personal invitation by the community health agents; and (iii)
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24 142 invitation by nurses in the school-based health promotion program. The study sample size (7,935
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26 143 individuals) was calculated to detect differences of 5% in HPV prevalence between regions with
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28 144 a power of 80% and an alpha error of 5% for women using an estimate of 30% [22] for the
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30 145 prevalence of HPV in Brazil. This sample size will allow an estimate with an error between 1.6%
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32 146 and 5.7%, according to sex.

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35 147 The sample size will be purposely equal in all regions (1,587 individuals) to maximize
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37 148 the diversity in less populated areas and will be standardized by the Brazilian population of each
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39 149 region during analyses.

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3 154 Participants will be asked to answer questions regarding age, gender, race/skin color,
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12 158 Smoking, alcohol and other drugs
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14 159 Questions concerning current and past smoking habits, including quantity and frequency,
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16 160 will be asked of the participants. Moreover, the interview includes questions about the use of
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18 161 alcohol and other drugs such as marijuana, cocaine, ecstasy, crack, and the abuse of morphine or
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20 162 other licit and illicit drugs.
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26 164 Sexual and reproductive health
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28 165 Participants will be asked to supply details about their age at first sexual intercourse, their
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30 166 involvement in same sex relationships, their use of condoms, the number of sexual partners, their
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32 167 sexual practices and the presence of any genital symptoms during or after sexual intercourse such
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34 168 as pain or bleeding.
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37 169 Their age of menarche, the number of pregnancies and deliveries, the number of
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39 170 abortions, their use of contraceptive methods and the occurrence of sexually transmitted
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41 171 infections will be asked of participants to evaluate their sexual health. All suspicious HPV-
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43 172 related lesions will be photographed for future evaluation.
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48 174 Knowledge about HPV, vaccination and screening tests
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50 175 We will measure how much participants know about HPV and vaccination (when and
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52 176 who must receive the vaccine). The questionnaire is based on Saulle et al [23]. Moreover,
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3 177 participants will be asked if they know about Pap smears, if they have already received the exam
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5 178 and if so, if any alteration was ever observed.
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10 180 HPV frequency and type
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12 181 We will measure the proportion of the sample that is infected by HPV, and we will look
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14 182 at the prevalence of high-risk types and the most prevalent types in the genital and oropharyngeal
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16 183 sites. Additionally, we will investigate the presence of chronic lesions (lasting 15 days or more)
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18 184 in the oral cavity that are related to the presence of HPV.
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22 186 **Outcomes**

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26 187 The nationwide prevalence of HPV in the genitals is the major outcome. Secondary
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28 188 outcomes include the prevalence of specific HPV types, the comparison of prevalence between
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30 189 Brazilian regions, and the prevalence of HR-HPVs and LR-HPVs.
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34 191 **Procedures**

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37 192 All individuals will respond to a standardized interview based on validated instruments
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39 193 [23–26]. After the interview, samples from the oral cavity and cervical or penile/scrotal sites will
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41 194 be collected from each participant.
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44 195 An online platform for data entry will be used by primary care professionals to add
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46 196 participant data, biological sample information and photographs. The same platform will be used
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48 197 for study process control and to allow availability of the results to primary care health
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50 198 professionals. Participants will have access to an external webpage where they will be able find
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52 199 information about their results, as protected by a password provided during interview. In cases in
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3 200 which high-risk HPV infection is detected, those participants will be asked to return to the
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5 201 primary care unit to be informed about the result.
6

7
8 202 Oral samples will be obtained through mouthwash and gargle cycles by using 10 ml of a
9
10 203 standardized commercial mouthwash. The samples will be obtained in 3 cycles of 5 seconds each
11
12 204 and will be stored in a 15-ml Falcon tube identified with a five digit number bar code and the
13
14 205 date of collection.
15

16
17 206 Cervical samples will be obtained using the Digene[®] HC2 DNA Collection kit (Qiagen)
18
19 207 and placed in 1 ml of Specimen Transport Medium (STM), according to the manufacturer's
20
21 208 instructions.
22

23
24 209 Penile (penile shaft, glans penis/coronal sulcus) and scrotum samples will be obtained
25
26 210 using a Dacron swab (Qiagen) previously moistened in sterile saline solution. Collection will be
27
28 211 performed by intensely pressing and rubbing the swab in the epithelium via self-collection under
29
30 212 supervision of the primary care health professional and after instruction regarding the procedure.
31
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33 213 All biological samples will be maintained at room temperature (15°C to 25°C) according to
34
35 214 manufacturer's instructions and transported to a central lab in Porto Alegre to start the specimen
36
37 215 processing. The temperature will be monitored with a data logger. Figure 1 presents the general
38
39 216 logistics for the nationwide multicenter collection of data and specimens.
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41

42 217 Individuals who present visible lesions on the genitals or in the oral cavity at the time of
43
44 218 recruitment will be asked to grant permission for a picture to be taken of the lesion.
45
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47 219

48 49 220 Specimen processing 50

51 221 All samples will be treated as biohazardous material, and all specimen handling will be
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53 222 performed in a biosafety cabinet. In the laboratory, specimens will be immediately aliquoted.
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3 223 One aliquot will be maintained at -80°C as a backup. Another aliquot will be processed for DNA
4
5 224 extraction from the STM medium using magnetic beads for isolation and purification using the
6
7 225 automatized extraction platform (MagNA Pure LC 2.0, Roche Molecular Systems)), after
8
9 226 exposed to an enzymatic treatment with proteinase K, following the manufacturer's instruction.

10
11
12 227 HPV detection and typing will be done using the Linear Array[®] HPV Genotyping Test
13
14 228 (Roche Diagnostics), which is based on PCR amplification (450-bp fragment of the polymorphic
15
16 229 region of the L1 gene of HPV), followed by specific hybridization and colorimetric detection,
17
18 230 according to the manufacturer's instructions. The test detects HR-HPVs (16, 18, 31, 33, 35, 39,
19
20 231 45, 51, 52, 56, 58, 59 and 68) and LR-HPVs (6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69,
21
22 232 70, 71 72, 73, 81, 82, 83, 84, IS39 and CP6198), for a total of 37 types. The amplification of
23
24 233 human β -globin (biotinylated primers GH20 and PC04, 268-bp fragment) is used as an internal
25
26 234 control for the PCR. To ensure reproducibility of LA, an automated AutoBlot instrument
27
28 235 (Fujirebio) was used for hybridization and wash steps. Real-time PCR using the TaqMan system
29
30 236 for HPV type 52 will be performed to confirm the results obtained by Roche's test as a
31
32 237 combination of HPV types 52, 33, 35 and 58.
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40 239 **Data analyses and statistics**

41
42 240 Categorical variables will be summarized using absolute frequencies and percentage,
43
44 241 while continuous variables will be analyzed using means and standard error. Chi-squared tests
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46 242 and Fisher's exact tests will be used to compare proportions, and t-tests or Mann-Whitney non-
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48 243 parametric tests will be used for continuous variables.
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3 244 The association between HPV infection and social and behavioral variables will be
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5 245 defined by calculating crude and adjusted prevalence ratios. Confounding variables will be
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7
8 246 evaluated using modified Poisson regression analysis [27].
9

10 247 To maximize diversity across regions, we have divided the sample size into equal
11
12 248 numbers among regions. To adjust the distribution of the sample to the study population, we will
13
14 249 standardize the measures by the size of the population in each capital within the age range and by
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16
17 250 sex.
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19 251

21 252 **Monitoring and quality control**

23
24 253 The data quality will be validated by routine practices. A pilot study was performed to
25
26 254 identify and correct problems in data collection, sample transportation, procedures and
27
28 255 instruments. All health professionals involved in the research are trained and certified by the
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30
31 256 coordinator team using simulated interview and sample collection according to the study
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33 257 protocol, as described in the POP-Brazil operations manuals.
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35 258 Data and specimen collection are periodically evaluated by reports generated by the data
36
37 259 system, including information about the number of participants and the number of refusals, and
38
39 260 the quality of the samples collected. Those reports will be distributed monthly to all people
40
41
42 261 involved in data collection.
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44 262 Moreover, each unit will receive at least one *in loco* monitoring visit, which will be done
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46 263 by research staff supervisors to evaluate protocol compliance and provide recertification.
47
48 264 Supervisors will observe techniques utilizing predefined check lists and recorded interviews.
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51 265 The biological sample tracking is done through the web-based data platform, and time,
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54 266 temperature and sample conditions are evaluated on a daily basis.
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3 267 To access the reliability of the interviews, a subsample will be obtained in duplicate. A
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5 268 resampling will be done in 10% of the interviews using phone calls to access the coefficient of
6
7 269 variation and systematic differences between data. Figure 2 presents the steps that will be taken
8
9
10 270 for quality assurance. The reliability of HPV detection will be accessed by double processing a
11
12 271 random subsample of the biological genital samples by the Centers for Disease Control and
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14 272 Prevention.
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16 273

19 274 **DISCUSSION**

21 275 This is the first Brazilian nationwide study to determine HPV prevalence and the social,
22
23 276 demographic and behavioral factors related to HPV infection. Critical analysis of the study
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25 277 results will contribute to epidemiological knowledge of the distribution of HPV strains across
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27 278 geographical regions and will establish a baseline to evaluate the impact of the Brazilian
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29 279 Vaccination Program.
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32
33 280 There are some limitations of this study. The sampling is not random. Brazil is a large
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35 281 country, and due to logistic reasons, the sampling is restricted to state capitals in public health
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37 282 units selected by the local government. To minimize selection bias, in addition to recruiting
38
39 283 participants that voluntarily come to the health unit, we will also recruit at schools, through the
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41 284 list of patients of the selected health units, and by personal invitation by community health
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43 285 agents in households. We are also only recruiting participants in the public health system, whom
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45 286 account for approximately 70% of the overall Brazilian population; therefore, we cannot draw
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47 287 inferences about people who exclusively use the private sector. In Brazil, the private sector is
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49 288 mainly used by social classes A and B, but it is important to highlight that for some services such
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51 289 as vaccination, the Public Health System covers virtually 100% of the population.
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3 290 The strengths of the study are the establishment of a baseline for future comparison of
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5 291 HPV prevalence, the nationwide sampling, including a large and diverse population and the use
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7 292 of central laboratory and techniques for data quality monitoring. Additionally, we will collect
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10 293 data on sexual behaviors and other STIs, making it possible to pursue some explanation
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12 294 regarding differences in prevalence according to specific groups of participants.

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14 295 Health managers, based on these data, will be able to compare HPV prevalence in
15
16 296 different regions according to population characteristics; it will be possible to support the
17
18 297 development of strategies to promote HPV vaccination and to develop strategies for prevention,
19
20 298 taking into account regional differences. Moreover, these data will serve as a baseline for future
21
22 299 comparisons and will allow monitoring of the effectiveness of the National HPV Vaccination
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24 300 Program.

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30 302 **ETHICS AND DISSEMINATION**

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33 303 Before starting the study, this project was submitted to the Research Ethical Committee
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35 304 of the proposing institution (Hospital Moinhos de Vento). Following approval, agreements were
36
37 305 made by all co-participant centers and submitted to their corresponding ethical committees.

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40 306 Individuals must voluntarily agree to participate and must sign the free and informed
41
42 307 consent form. The study follows the ethical recommendations of Resolutions 347/05, 441/11 and
43
44 308 466/12 of the National Health Council.

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47 309 In cases of no infection or infection with a low-risk HPV type, participants will be
48
49 310 informed of negative results via web access. Conversely, participants infected with high-risk
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51 311 HPV types will be advised to go to the health care unit for their results, where they will be

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3 312 oriented about the meaning of the result and instructed about follow-up according to national
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5 313 guidelines.
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7
8 314 Preliminary results will be presented as poster and/or oral sections in national and
9
10 315 international congresses, and the final results will be submitted for publishing in peer-reviewed
11
12 316 open-access journals. The results will also be presented in a personal meeting to the Ministry of
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14 317 Health of Brazil.
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3 386 **AUTHOR CONTRIBUTIONS**
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5 387 EMW conceived of the project and participated in all phases of the manuscript preparation; ASB,
6
7 388 AGM, LSH, MFAS, JH, FM, LLV, CMD and MB participated in the protocol development; and
8
9 389 JC and LLV participated in the laboratory protocol development and helped write the
10
11 manuscript. All authors reviewed the final version of the manuscript.
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18

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20
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22
23 395 Ministry of Health of Brazil, with funding participation of the Pan-American Health
24
25 396 Organization.
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31 398 **COMPETING INTERESTS**
32

33 399 ASB, FMAS, CMD and AGM work for the Ministry of Health of Brazil. The other authors
34
35 400 declare no conflict of interest. LLV is a consultant for Merck for the HPV quadrivalent vaccine
36
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42 403 **ACKNOWLEDGMENTS**
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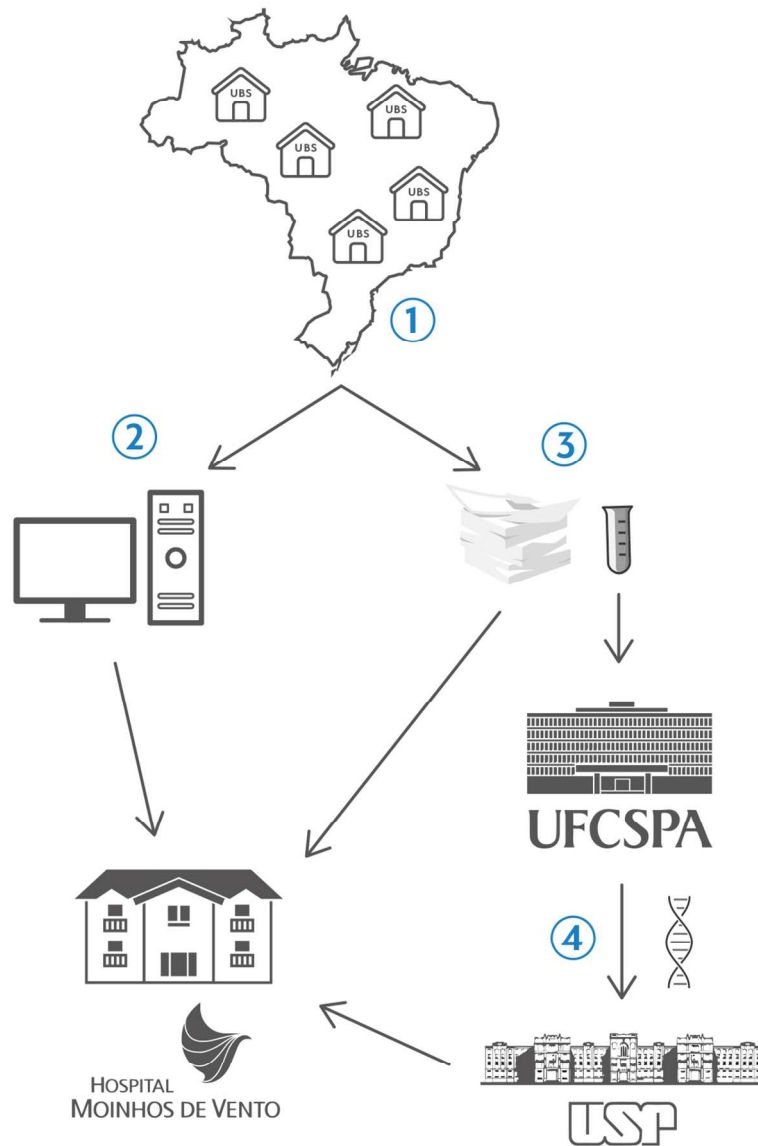
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47
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49
50 407 helpful suggestions in developing the study protocol and quality control.
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3 409 **FIGURE LEGENDS**
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5 410 **Figure 1.** Workflow of the study. 1. Data are collected in public health units from all 27
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7 411 Brazilian capitals; 2. Data entry into the web-based system is performed by primary care
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9 412 professionals and is monitored by the main research team in Porto Alegre (Hospital Moinhos de
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11 413 Vento); 3. Paper forms are sent to Porto Alegre along with biological samples, which are
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13 414 processed for DNA extraction (UFCSPA); and 4. Extracted DNA is sent to São Paulo (USP) for
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15 415 HPV detection and typing. Finally, the results are included in the web-based system and are
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17 416 made available for the primary care professionals and participants using a code number and
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19 417 password.
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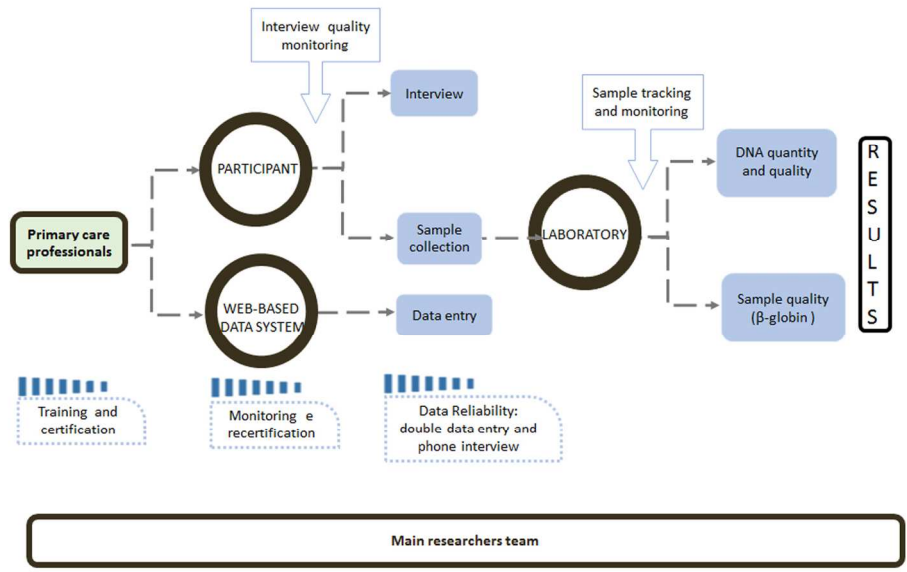
24 418 **Figure 2.** Description of the study's major quality control steps during each phase of the study.
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Workflow of the study. 1. Data are collected in Public Health Units from all 27 Brazilian capitals; 2. Data entry on the web-based system are performed by primary care professionals and are monitored by main research team in Porto Alegre (Hospital Moinhos de Vento); 3. Paper forms are sent to Porto Alegre along with biological samples, which are processed for DNA extraction (UFCSPA); 4. Extracted DNA are sent to São Paulo (USP) for HPV detection and typing. Finally, results are included on the web-based system and became available for primary care professionals and participants.

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Description of the study's major quality control steps during each phase of the study.

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BMJ Open

POP-Brazil Study Protocol: a nationwide cross-sectional evaluation of the prevalence and genotype distribution of human papillomavirus (HPV) in Brazil

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Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Sexual health, Public health, Infectious diseases
Keywords:	nationwide study, HPV infection, anogenital, prevalence, Brazil

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Manuscripts

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3 1 **POP-Brazil Study Protocol: A nationwide cross-sectional evaluation of the prevalence and**
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5 2 **genotype distribution of human papillomavirus (HPV) in Brazil**
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For peer review only

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3 30 **ABSTRACT**
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5 31 **Introduction:** Human papillomavirus (HPV) is associated with the development of genital warts
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7 32 and different types of cancer, including virtually all cervical cancers and a considerable number
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9 33 of penile, anal and oropharyngeal cancers. Data regarding the prevalence of HPV infection in
10
11 34 Brazil are limited and fragmented. We aim to determine HPV prevalence in sexually active
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13 35 women and men from 16 to 25 years old and to investigate regional differences in virus
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15 36 prevalence and types. **Methods and analysis:** This is a nationwide, multi-centric, cross-
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17 37 sectional, prospective study that will include participants from 16 to 25 years old from all
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19 38 Brazilian capital cities. Recruitment will occur in primary health units by trained health
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21 39 professionals who will be responsible for collecting biological samples and interviewing the
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23 40 volunteers. After signing informed consent, all participants will answer a questionnaire that will
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25 41 collect sociodemographic and behavioral data. All samples will be processed in a certified
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27 42 central laboratory, and strict quality control will be performed by many different procedures,
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29 43 including double data entry, training and certification of primary care health professionals
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31 44 responsible for data collection, simulation of interviews and auditing and monitoring of visits.
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33 45 The sample size will be standardized based on the population distribution of each capital using
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35 46 SAS[®] and R statistical software. **Ethics and dissemination:** The project was approved by the
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37 47 Research Ethics Committee of the main institution and the corresponding ethics committees of
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39 48 the recruitment sites. This will be the first Brazilian nationwide study to determine overall HPV
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41 49 prevalence and to examine regional differences and social, demographic and behavioral factors
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43 50 related to HPV infection. Critical analysis of the study results will contribute to epidemiological
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45 51 knowledge and will set a baseline for future evaluation of the impact of the National HPV
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47 52 Vaccination Program.
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45 54 **Keywords:** HPV infection, prevalence, nationwide study, Brazil, anogenital
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910 56 **Strengths and limitations of this study**
1112 57 • Representativeness of all regions of Brazil.
1314 58 • Establishment of a baseline to evaluate the effectiveness of the HPV vaccination program
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17 59 in Brazil.
1819 60 • Recruitment done in primary care units, inviting all people within the age range living in
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21 61 the area and not over representing risk factor groups.
2223 62 • Only the population that uses the primary care units from the public health system will be
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26 63 included, but that consists of 71% of the Brazilian population.
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65 INTRODUCTION

66 According to the World Health Organization (WHO), more than 528,000 incident cases
67 and approximately 270,000 deaths due to cervical cancer occur annually worldwide, 85% of
68 which occur in low- and middle-income countries [1]. Virtually all these cancers are preceded by
69 infection with high-risk oncogenic human papillomaviruses (HR-HPVs) [2], and HPV types 16
70 and 18 are associated with 70% of these cases [3,4] In Brazil, the National Institute of Cancer
71 estimated that there were 16,340 new cervical carcinoma cases in 2016, with a 33.23% mortality
72 rate [5].

73 HR-HPVs are also responsible for 63% of penile cancers, 89% of anal cancers and 72%
74 of oropharyngeal cancers in men [6,7]. Indeed, these viruses are now recognized as the major
75 cause of the observed rising incidence rates of oropharyngeal squamous cell carcinoma; indeed,
76 the primary risk factor for developing this cancer is oral HPV infection [8].

77 Most sexually active individuals will be infected by HPV at some point in their lives
78 [9,10]. In Brazil, the prevalence of HPV infection in women with normal cervical cytology
79 varies from 2.3% to 55.4% [11,12]. Considerable variability in HPV prevalence rates for other
80 body sites, such as the penile epithelium and the oral cavity, are also observed in Brazil [11,12].

81 These data originated from studies that were heterogeneous with regard to methodology
82 and/or the study population. In addition, virtually all studies to date were geographically
83 restricted [13–17] and/or focused on specific groups of individuals, such as HIV-positive,
84 pregnant and immunosuppressed patients [18–20]. Moreover, some of those studies recruited
85 participants from clinics specializing in sexually transmitted infections, resulting in selection bias
86 and inflation of the estimated prevalence [13–20].

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3 87 Vaccination against HPV is being performed in many countries, and it is a method of
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5 88 primary prevention of cancer and HPV infection [9]. Since 2014, the Brazilian Ministry of
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7 89 Health has provided a quadrivalent (types 6, 11, 16 and 18) vaccine for women from 9-14 years
8
9 90 old, with a schedule of 0 and 6 months. In January 2017, Brazil was the first country in Latin
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11 91 America to extend vaccination to men (11-14 years old) [21]. Baseline data of HPV prevalence
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13 92 before vaccinations are vital in order to establish the impact of vaccination on the distribution of
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15 93 HPV types.

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19 94 As far as we know, this is the first study presenting a nationwide scope with uniform
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21 95 methodology to evaluate the Brazilian prevalence of HPV and its types, as well as to compare
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23 96 the prevalence among specific geographical regions that present marked differences in social,
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25 97 demographic, cultural and economic characteristics. In addition, the study will describe the
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27 98 social, demographic, economic and behavioral factors associated with HPV positivity.
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32 33 100 **OBJECTIVES**

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35 101 The primary objective of the study is to determine the prevalence of HPV in women and
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37 102 men aged 16-25 years in Brazil, evaluating the most prevalent types and possible differences
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39 103 between regions and risk factors associated with positivity to establish a baseline for evaluation
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41 104 of vaccine effectiveness.
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46 47 106 **Secondary objectives**

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49 107 • To determine the prevalence of HPV, high-risk types, genital warts and the
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51 108 associated sexual behaviors;
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3 109 • To evaluate the co-infection of HPV-HIV in the young population that uses the
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5 110 public health system;
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8 111 • To investigate beliefs and knowledge about HPV infection and vaccination in a
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10 112 young population;
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12 113 • To evaluate the association of other sexually transmitted infections (STIs),
13
14 114 such as syphilis, with the presence of HPV;
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17 115 • To evaluate the herd effect in a sample of unvaccinated men.
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21 22 117 **METHODS AND ANALYSIS**

23 24 118 25 26 119 **Design**

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29 120 We will use a cross-sectional, nationwide, multi-centric design to establish a baseline
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31 121 dataset that will enable prevalence comparison over time.
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33 122 34 35 123 **Recruitment and sample size**

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38 124 Beginning in January 2017, sexually active women and men from 16 to 25 years old who
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40 125 use the public health system in all 26 Brazilian capitals plus the Federal District of Brasilia
41
42 126 across the five geographical regions will be recruited through public primary care units

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45 127 Because endocervical collection is not recommended during pregnancy in Brazil and/or
46
47 128 to avoid selection bias, the following exclusion criteria will be applied: pregnant women, those
48
49 129 who delivered a baby in the last 3 months, those who have undergone a hysterectomy or
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51 130 trachelectomy and those who have ever had cervical intraepithelial neoplasia grade 2 or higher
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54 131 will be considered ineligible for the study.
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3 132 There will be no exclusion based on HPV vaccination status, but we will investigate
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5 133 whether the participants have been vaccinated. Since the vaccination program was launched in
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7 134 2014 for women who were 9-13 years old and our study population is among 16-25-year-olds,
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9
10 135 we believe that most of the volunteers will be not have been vaccinated.

11
12 136 The primary care units in each city were selected based on their representativeness of the
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14 137 health districts and based on their resources to collect and store oral samples. Most of these units
15
16 138 have a defined territory and a list of all people within the age range. When the list is available, all
17
18 139 participants will be invited. When the list is not available, participants will be recruited by
19
20 140 different approaches: (i) eligible individuals who came to the unit for any reason not associated
21
22 141 with the main study's outcome; (ii) personal invitation by the community health agents; and (iii)
23
24 142 invitation by nurses in the school-based health promotion program. The study sample size (7,935
25
26 143 individuals) was calculated to detect differences of 5% in HPV prevalence between regions with
27
28 144 a power of 80% and an alpha error of 5% for women using an estimate of 30% [22] for the
29
30 145 prevalence of HPV in Brazil. This sample size will allow an estimate with an error between 1.6%
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32 146 and 5.7%, according to sex.

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35 147 The sample size will be purposely equal in all regions (1,587 individuals) to maximize
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37 148 the diversity in less populated areas and will be standardized by the Brazilian population of each
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39 149 region during analyses.

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43 44 45 151 **Measurements**

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51 153 Sociodemographic variables
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3 154 Participants will be asked to answer questions regarding age, gender, race/skin color,
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5 155 household characteristics, relationship status, educational level, occupational status, last-month
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7 156 family income and the number of people dependent on this income.
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10 157
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12 158 Smoking, alcohol and other drugs
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14 159 Questions concerning current and past smoking habits, including quantity and frequency,
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16 160 will be asked of the participants. Moreover, the interview includes questions about the use of
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18 161 alcohol and other drugs such as marijuana, cocaine, ecstasy, crack, and the abuse of morphine or
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20 162 other licit and illicit drugs.
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26 164 Sexual and reproductive health
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28 165 Participants will be asked to supply details about their age at first sexual intercourse, their
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30 166 involvement in same sex relationships, their use of condoms, the number of sexual partners, their
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32 167 sexual practices and the presence of any genital symptoms during or after sexual intercourse such
33
34 168 as pain or bleeding.
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37 169 Their age of menarche, the number of pregnancies and deliveries, the number of
38
39 170 abortions, their use of contraceptive methods and the occurrence of sexually transmitted
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41 171 infections will be asked of participants to evaluate their sexual health. All suspicious HPV-
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43 172 related lesions will be photographed for future evaluation.
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48 174 Knowledge about HPV, vaccination and screening tests
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50 175 We will measure how much participants know about HPV and vaccination (when and
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52 176 who must receive the vaccine). The questionnaire is based on Saulle et al [23]. Moreover,
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3 177 participants will be asked if they know about Pap smears, if they have already received the exam
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5 178 and if so, if any alteration was ever observed.
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10 180 HPV frequency and type

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12 181 We will measure the proportion of the sample that is infected by HPV, and we will look
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14 182 at the prevalence of high-risk types and the most prevalent types in the genital and oropharyngeal
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16 183 sites. Additionally, we will investigate the presence of chronic lesions (lasting 15 days or more)
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18 184 in the oral cavity that are related to the presence of HPV.
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22 186 **Outcomes**

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26 187 The nationwide prevalence of HPV in the genitals is the major outcome. Secondary
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28 188 outcomes include the prevalence of specific HPV types, the comparison of prevalence between
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30 189 Brazilian regions, and the prevalence of HR-HPVs and LR-HPVs.
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34 191 **Procedures**

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37 192 All individuals will respond to a standardized interview based on validated instruments
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39 193 [23–26]. After the interview, samples from the oral cavity and cervical or penile/scrotal sites will
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41 194 be collected from each participant.
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44 195 An online platform for data entry will be used by primary care professionals to add
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46 196 participant data, biological sample information and photographs. The same platform will be used
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48 197 for study process control and to allow availability of the results to primary care health
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50 198 professionals. Participants will have access to an external webpage where they will be able find
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52 199 information about their results, as protected by a password provided during interview. In cases in
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3 200 which high-risk HPV infection is detected, those participants will be asked to return to the
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5 201 primary care unit to be informed about the result.
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8 202 Oral samples will be obtained through mouthwash and gargle cycles by using 10 ml of a
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10 203 standardized commercial mouthwash. The samples will be obtained in 3 cycles of 5 seconds each
11
12 204 and will be stored in a 15-ml Falcon tube identified with a five digit number bar code and the
13
14 205 date of collection.
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16
17 206 Cervical samples will be obtained using the Digene[®] HC2 DNA Collection kit (Qiagen)
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19 207 and placed in 1 ml of Specimen Transport Medium (STM), according to the manufacturer's
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21 208 instructions.
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24 209 Penile (penile shaft, glans penis/coronal sulcus) and scrotum samples will be obtained
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26 210 using a Dacron swab (Qiagen) previously moistened in sterile saline solution. Collection will be
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28 211 performed by intensely pressing and rubbing the swab in the epithelium via self-collection under
29
30 212 supervision of the primary care health professional and after instruction regarding the procedure.
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33 213 All biological samples will be maintained at room temperature (15°C to 25°C) according to
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35 214 manufacturer's instructions and transported to a central lab in Porto Alegre to start the specimen
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37 215 processing. The temperature will be monitored with a data logger. Figure 1 presents the general
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39 216 logistics for the nationwide multicenter collection of data and specimens.
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42 217 Individuals who present visible lesions on the genitals or in the oral cavity at the time of
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44 218 recruitment will be asked to grant permission for a picture to be taken of the lesion.
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48 49 220 Specimen processing 50

51 221 All samples will be treated as biohazardous material, and all specimen handling will be
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53 222 performed in a biosafety cabinet. In the laboratory, specimens will be immediately aliquoted.
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3 223 One aliquot will be maintained at -80°C as a backup. Another aliquot will be processed for DNA
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5 224 extraction from the STM medium using magnetic beads for isolation and purification using the
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8 225 automatized extraction platform (MagNA Pure LC 2.0, Roche Molecular Systems)), after
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10 226 exposed to an enzymatic treatment with proteinase K, following the manufacturer's instruction.

11
12 227 HPV detection and typing will be done using the Linear Array[®] HPV Genotyping Test
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14 228 (Roche Diagnostics), which is based on PCR amplification (450-bp fragment of the polymorphic
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17 229 region of the L1 gene of HPV), followed by specific hybridization and colorimetric detection,
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19 230 according to the manufacturer's instructions. The test detects HR-HPVs (16, 18, 31, 33, 35, 39,
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21 231 45, 51, 52, 56, 58, 59 and 68) and LR-HPVs (6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 66, 67, 69,
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24 232 70, 71 72, 73, 81, 82, 83, 84, IS39 and CP6198), for a total of 37 types. The amplification of
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26 233 human β -globin (biotinylated primers GH20 and PC04, 268-bp fragment) is used as an internal
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28 234 control for the PCR. To ensure reproducibility of LA, an automated AutoBlot instrument
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31 235 (Fujirebio) was used for hybridization and wash steps. Real-time PCR using the TaqMan system
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33 236 for HPV type 52 will be performed to confirm the results obtained by Roche's test as a
34
35 237 combination of HPV types 52, 33, 35 and 58.

36 37 38 39 40 239 **Data analyses and statistics**

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42 240 Categorical variables will be summarized using absolute frequencies and percentage,
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44 241 while continuous variables will be analyzed using means and standard error. Chi-squared tests
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47 242 and Fisher's exact tests will be used to compare proportions, and t-tests or Mann-Whitney non-
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49 243 parametric tests will be used for continuous variables.

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3 244 The association between HPV infection and social and behavioral variables will be
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5 245 defined by calculating crude and adjusted prevalence ratios. Confounding variables will be
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8 246 evaluated using modified Poisson regression analysis [27].
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10 247 To maximize diversity across regions, we have divided the sample size into equal
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12 248 numbers among regions. To adjust the distribution of the sample to the study population, we will
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14 249 standardize the measures by the size of the population in each capital within the age range and by
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17 250 sex.
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21 252 **Monitoring and quality control**

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24 253 The data quality will be validated by routine practices. A pilot study was performed to
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26 254 identify and correct problems in data collection, sample transportation, procedures and
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28 255 instruments. All health professionals involved in the research are trained and certified by the
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31 256 coordinator team using simulated interview and sample collection according to the study
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33 257 protocol, as described in the POP-Brazil operations manuals.
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35 258 Data and specimen collection are periodically evaluated by reports generated by the data
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37 259 system, including information about the number of participants and the number of refusals, and
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39 260 the quality of the samples collected. Those reports will be distributed monthly to all people
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41
42 261 involved in data collection.
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44 262 Moreover, each unit will receive at least one *in loco* monitoring visit, which will be done
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46 263 by research staff supervisors to evaluate protocol compliance and provide recertification.
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48 264 Supervisors will observe techniques utilizing predefined check lists and recorded interviews.
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51 265 The biological sample tracking is done through the web-based data platform, and time,
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54 266 temperature and sample conditions are evaluated on a daily basis.
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3 267 To access the reliability of the interviews, a subsample will be obtained in duplicate. A
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5 268 resampling will be done in 10% of the interviews using phone calls to access the coefficient of
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7 269 variation and systematic differences between data. Figure 2 presents the steps that will be taken
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9 270 for quality assurance. The reliability of HPV detection will be accessed by double processing a
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11 271 random subsample of the biological genital samples by the Centers for Disease Control and
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13 272 Prevention.
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19 274 **Patient and Public Involvement**

21 275 Patients and public were not involved in the study design and/or protocol. In
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23 276 cases of no infection or infection with a low-risk HPV type, participants will be informed of
24
25 277 negative results via web access. Conversely, participants infected with high-risk HPV types will
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27 278 be advised to go to the health care unit for their results, where they will be oriented about the
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29 279 meaning of the result and instructed about follow-up according to national guidelines.
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33 280 Participants will be informed about the study results through study web site.
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40 283 **DISCUSSION**

41
42 284 This is the first Brazilian nationwide study to determine HPV prevalence and the social,
43
44 285 demographic and behavioral factors related to HPV infection. Critical analysis of the study
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46 286 results will contribute to epidemiological knowledge of the distribution of HPV strains across
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48 287 geographical regions and will establish a baseline to evaluate the impact of the Brazilian
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50 288 Vaccination Program.
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3 289 There are some limitations of this study. The sampling is not random. Brazil is a large
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5 290 country, and due to logistic reasons, the sampling is restricted to state capitals in public health
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7 291 units selected by the local government. To minimize selection bias, in addition to recruiting
8
9 292 participants that voluntarily come to the health unit, we will also recruit at schools, through the
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11 293 list of patients of the selected health units, and by personal invitation by community health
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13 294 agents in households. We are also only recruiting participants in the public health system, whom
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15 295 account for approximately 70% of the overall Brazilian population; therefore, we cannot draw
16
17 296 inferences about people who exclusively use the private sector. In Brazil, the private sector is
18
19 297 mainly used by social classes A and B, but it is important to highlight that for some services such
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21 298 as vaccination, the Public Health System covers virtually 100% of the population.
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26 299 The strengths of the study are the establishment of a baseline for future comparison of
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28 300 HPV prevalence, the nationwide sampling, including a large and diverse population and the use
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30 301 of central laboratory and techniques for data quality monitoring. Additionally, we will collect
31
32 302 data on sexual behaviors and other STIs, making it possible to pursue some explanation
33
34 303 regarding differences in prevalence according to specific groups of participants.
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38 304 Health managers, based on these data, will be able to compare HPV prevalence in
39
40 305 different regions according to population characteristics; it will be possible to support the
41
42 306 development of strategies to promote HPV vaccination and to develop strategies for prevention,
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44 307 taking into account regional differences. Moreover, these data will serve as a baseline for future
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46 308 comparisons and will allow monitoring of the effectiveness of the National HPV Vaccination
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48 309 Program.
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52 53 311 **ETHICS AND DISSEMINATION**

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3 312 Before starting the study, this project was submitted to the Research Ethical Committee
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5 313 of the proposing institution (Hospital Moinhos de Vento). Following approval, agreements were
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7 314 made by all co-participant centers and submitted to their corresponding ethical committees.
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9

10 315 Individuals must voluntarily agree to participate and must sign the free and informed
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12 316 consent form. The study follows the ethical recommendations of Resolutions 347/05, 441/11 and
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14 317 466/12 of the National Health Council.
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17 318 Preliminary results will be presented as poster and/or oral sections in national and
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19 319 international congresses, and the final results will be submitted for publishing in peer-reviewed
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21 320 open-access journals. The results will also be presented in a personal meeting to the Ministry of
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23 321 Health of Brazil.
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3 391 **AUTHOR CONTRIBUTIONS**
4

5 392 EMW conceived of the project and participated in all phases of the manuscript preparation; ASB,
6
7 393 AGM, LSH, MFAS, JH, FHN, FM, LLV, CMD and MB participated in the protocol
8
9
10 394 development; and JC and LLV participated in the laboratory protocol development and helped
11
12 395 write the manuscript. All authors reviewed the final version of the manuscript.
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16
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22
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25 401 Organization.
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31 403 **COMPETING INTERESTS**
32

33 404 ASB, FMAS, CMD and AGM work for the Ministry of Health of Brazil. The other authors
34
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36
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42 408 **ACKNOWLEDGMENTS**
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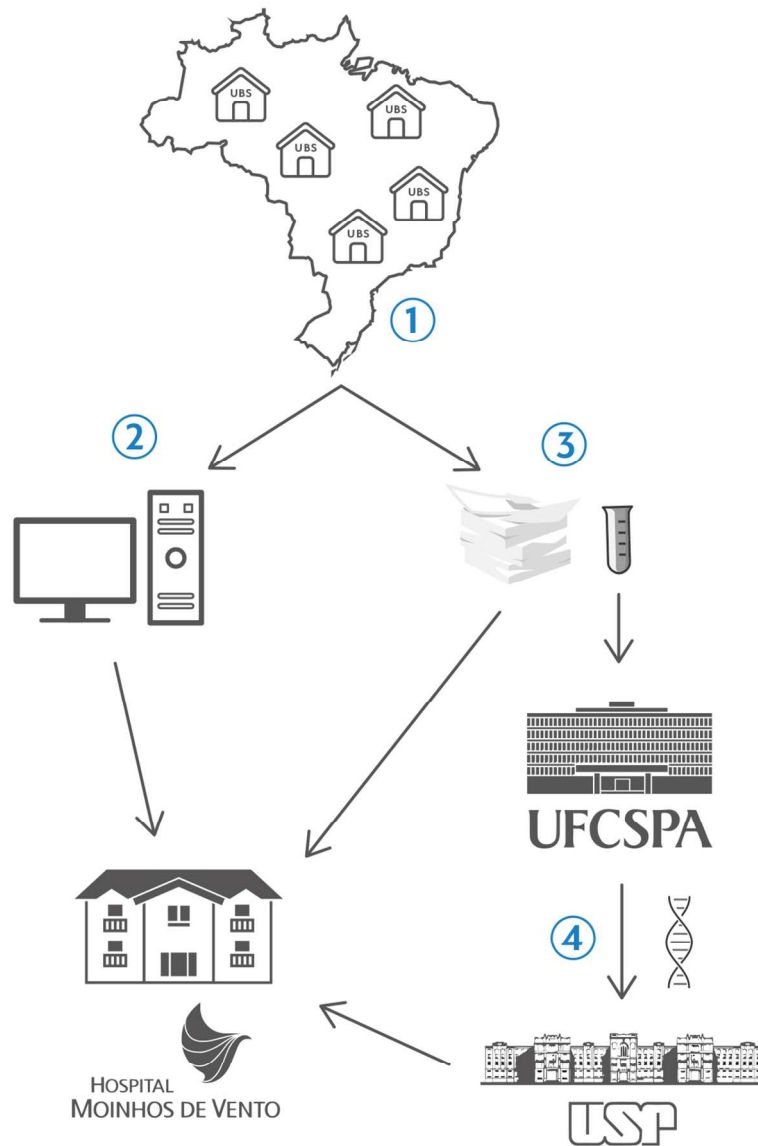
44 409 We would like to thank Julian Peto and Claire Gilham from the London School of Hygiene and
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50 412 helpful suggestions in developing the study protocol and quality control.
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3 414 **FIGURE LEGENDS**
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5 415 **Figure 1.** Workflow of the study. 1. Data are collected in public health units from all 27
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7 416 Brazilian capitals; 2. Data entry into the web-based system is performed by primary care
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9 417 professionals and is monitored by the main research team in Porto Alegre (Hospital Moinhos de
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11 418 Vento); 3. Paper forms are sent to Porto Alegre along with biological samples, which are
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13 419 processed for DNA extraction (UFCSPA); and 4. Extracted DNA is sent to São Paulo (USP) for
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15 420 HPV detection and typing. Finally, the results are included in the web-based system and are
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17 421 made available for the primary care professionals and participants using a code number and
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19 422 password.
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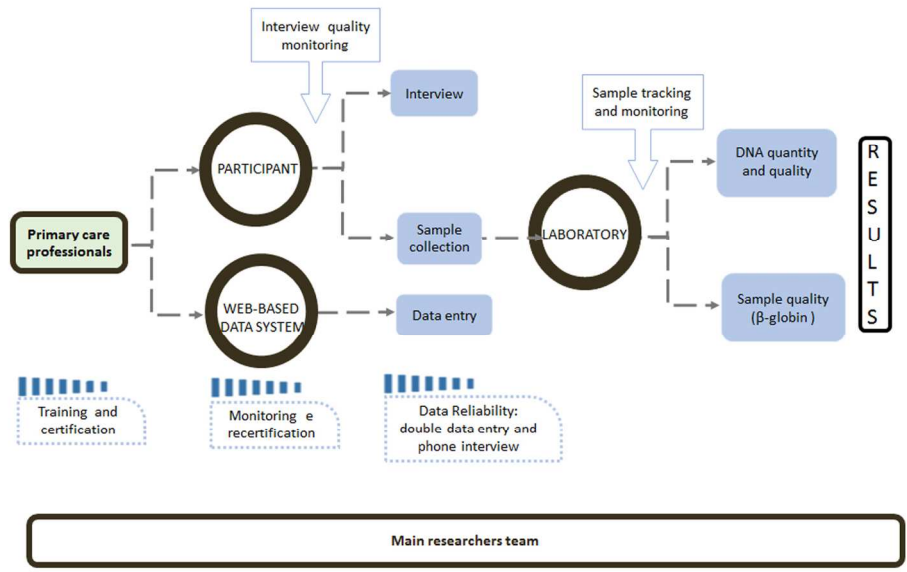
24 423 **Figure 2.** Description of the study's major quality control steps during each phase of the study.
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Workflow of the study. 1. Data are collected in Public Health Units from all 27 Brazilian capitals; 2. Data entry on the web-based system are performed by primary care professionals and are monitored by main research team in Porto Alegre (Hospital Moinhos de Vento); 3. Paper forms are sent to Porto Alegre along with biological samples, which are processed for DNA extraction (UFCSPA); 4. Extracted DNA are sent to São Paulo (USP) for HPV detection and typing. Finally, results are included on the web-based system and became available for primary care professionals and participants.

106x143mm (300 x 300 DPI)

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Description of the study's major quality control steps during each phase of the study.

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view only