2019

Working

Laboratory protocols for the detection and genotyping of Cervical high-risk human papillomavirus (HR-HPV) among adult women living in N'Djamena the capital city of Chad.

PLOS One

Ralph-Sydney Mboumba Bouassa[1], Zita Aleyo Nodjikouambaye[2], Damtheou Sadjoli[3], Chatté Adawaye[4], Hélène Péré[5], David Veyer[5], Mathieu Matta[5], Leman Robin[5], Serge Tonen-Wolyec[6], Ali Mahamat Moussa[7], Donato Koyalta and Laurent Belec.[8], Laurent Bélec[5]

[1]Ecole Doctorale Régionale d'Infectiologie Tropicale de Franceville, Gabon and Laboratoire de Virologie, Hôpital Européen Georges Pompidou, and Faculté de Médecine Paris Descartes, Université Paris Descartes (Paris V), Sorbonne Paris Cité, Paris, France, [2]Ecole Doctorale Régionale d'Infectiologie Tropicale de Franceville, Gabon and Service de Gynécologie-Obstétrique, Hôpital de la Mère et de l'Enfant, and Cabinet Médical de Gynécologie Obstétrique "La Renaissance Plus," N'Djamena, Chad, [3]Service de Gynécologie-Obstétrique, Hôpital de la Mère et de l'Enfant, and Cabinet Médical de Gynécologie Obstétrique "La Renaissance Plus," N'Djamena, Chad and Faculté des Sciences de la Santé Humaine, N'Djamena, Chad, [4]Institut National Supérieur des Sciences et Techniques d'Abéché, Chad, [5]Laboratoire de Virologie, Hôpital Européen Georges Pompidou, and Faculté de Médecine Paris Descartes, Université Paris Descartes (Paris V), Sorbonne Paris Cité, Paris, France, [6]Ecole Doctorale Régionale d'Infectiologie Tropicale de Franceville, Gabon and Faculté de Médecine, Université de Bunia, Democratic Republic of the Congo and Faculté de Médecine et de Pharmacie, Université de Kisangani, Democratic Republic of the Congo, [7]Faculté des Sciences de la Santé Humaine, N'Djamena, Chad and Service de Gastro-entérologie, Hôpital Général de Référence Nationale, N'Djamena, Chad, [8]UNAIDS, N'Djamena, Chad

dx.doi.org/10.17504/protocols.io.wgefbte

MBOUMBA BOUASSA Ralph

ABSTRACT

Adult Chadian women from 5 of the 10 districts of N'Djamena, the capital city of Chad, were randomly selected for inclusion. Peer educators contacted adult women in community-churches or women association networks to participate to the survey and come to the clinic for women's sexual health "La Renaissance Plus", N'Djamena. Medical, socio-demographical and behavioral informations were collected. HPV DNA was detected and genotyped in endocervical swab using Anyplex™ II HPV28 genotyping test (Seegene, Seoul, South Korea).

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0217486

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Bouassa RM, Nodjikouambaye ZA, Sadjoli D, Adawaye C, Péré H, Veyer D, Matta M, Robin L, Tonen-Wolyec S, Moussa AM, Koyalta D, Belec L (2019) High prevalence of cervical high-risk human papillomavirus infection mostly covered by Gardasil-9 prophylactic vaccine in adult women living in N'Djamena, Chad. PLoS ONE 14(6): e0217486. doi: 10.1371/journal.pone.0217486

GUIDELINES

Protocol:

After signed the informed consent form, the selected women benefited from free HIV and hepatitis B (HBV) and C (HCV) testing, by multiplex HIV/HCV/HBsAg immunochromatographic rapid test (Biosynex, Strasbourg, France).

The [Original Equipment Manufacturer:Biotest Biotech Inc., Hangzhou, China, under the name HCV/HBSAg/HIV COMBO rapid test cassette (whole blood/serum/plasma); reference: ITHD-C43] consists in manually performed, visually interpreted, lateralflow, immunochromatographic RDT simultaneously detecting in 15 min human immunodeficiency virus (HIV)-1 and HIV-2and hepatitis C virus (HCV)- specific antibodies (Ab) (IgG and IgM) and hepatitis B virus (HBV) surface antigen (HBsAg) in serum, plasma and whole blood (venipuncture and fingerstick). The test uses synthetic antigens (gp41, gp36) able to detect antibodies against HIV-1 or HIV-2,

monoclonal antibody to HbsAg, as well as fusion recombinant multiepitope chimeric HCV protein containing structural (core) and nonstructural (NS3, NS4, and NS5) HCV antigens, which are all bound to the solid phase membrane. Specimen presence is controlled by blood deposit assessment and migration control band. The HIV/HCV/HbsAg. Triplex is packaged individually containing all of the necessary components to perform the test.

HIV/HCV/HbsAg Triplex procedure:

One drop (50ml) of fingerstick whole blood or 50 ml of venipuncture whole blood or 25 ml of serum / plasma of the study participant is introduced in the suitable hole reserved for blood deposit. Then after, 4 drops of the test-provided buffer are added to the buffer hole on the test cassette. No more than 15 minutes are required before the test is read for HCV (band T1), HBV (band T2) and HIV (band T3) results.

Positive test were confirmed with gold-standard assays.

HIV, HBV or HCV positive women received adequate treatment and those negative for HBV and HCV, the clinician proposed them vaccination against HBV.

Sample collection procedure:

After completing the socio-demographic data collection questionnaire, a nurse performed cervicovaginal sampling using a flocked swab (Copan Diagnostic Inc., California, USA). Briefly, specimens for molecular testing were obtained by inserting the swab into the vaginal canal until the cervix mucosa, gently rotating 5 times and then removed and immediately placed into its container and frozen at -80°C before DNA extraction procedure. Finally, cervicovaginal swab were transported in frozen ice packs, to the virology laboratory of the hôpital Européen Georges Pompidou, Paris, France, for molecular analyses.

Total DNA extraction procedure:

DNA was extracted from cervical swab specimen using the DNeasyBlood and Tissue kit, as recommended by the manufacturer (Qiagen, Hilden, Germany).

Things to do before starting:

Equilibrate samples to room temperature (15–25°C).

Heat a water bath or heating block to 56°C for use in step 4.

Equilibrate Buffer AE or distilled water to room temperature for elution in step 11.

Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease have been prepared according to the manufacturer instructions.

1. Place the tip of the flocked swab specimen in a 1.5 ml tube containing 1ml of phosphate buffered saline (PBS). The 1.5ml tube containing the tip of the swab is vortexed to re-suspend cervicovaginal secretions in the PBS.

2. Pipet 20 µl QIAGEN Protease (or proteinase K) into the bottom of a 1.5 ml microcentrifuge tube.

3. Add 200 µl of the sample to the microcentrifuge tube.

4. Add 200 µl Buffer AL to the sample. Mix by pulse-vortexing for 15 s. To ensure efficient lysis, it is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution.

5. Incubate at 56°C for 10 min. DNA yield reaches a maximum after lysis for 10 min at 56°C. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

6. Add 200 µl ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 s. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

7. Carefully apply the mixture from step 6 to the Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the Mini spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate. Close each spin column to avoid aerosol formation during centrifugation.

8. Carefully open the Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the Mini spin column in a clean 2 ml collection tube (provided), and discard the collection tube containing the filtrate.

9. Carefully open the Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.

10. Recommended: Place the Mini spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min. This step helps to eliminate the chance of possible Buffer AW2 carryover.

11. Place the Mini spin column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the Mini spin column and add 200 µl Buffer AE.

12. Incubate at room temperature (15–25°C) for 1 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min.

After extraction, DNA was concentrated and eluted in 100 to 200µL of kit elution buffer before genotyping.

HPV detection and genotyping procedure:

The detection of HPV DNA and the distribution of genotypes were done using Anyplex™ II HPV28 detection test (Seegene, Seoul, South Korea). Anyplex™ II HPV28 detection test was performed as recommended by the manufacturer with 5µl of DNA in each of the two reaction mixtures (20 µl) with the primers A and B. According to the International Agency for Research on Cancer (IARC) nomenclature, Anyplex™ II HPV28 detection test distinguishes 28 HPV genotypes, including 13 high-risk types (HR-HPV -16, -18, -31, -33, -35, -39, -45,-51, -52, -56, -58, -59, and -68), 9 low-risk (LR) types (LR-HPV -6, -11, -40, -42, -43, -44,-53, -54 and -70) and then, 6 genotypes reported as possibly carcinogenic (HPV-26, -61, -66, -69, -73 and -82). The process is carried out in 2 reactions by taking advantage of the 5 dyes that can be resolved on the CFX96™ real-time PCR instrument (Bio-Rad, Marnes-la-Coquette, France).

The Anyplex TM II HPV28 Detection assay consists of two PCR reactions (A set and B set).

A set is a multiplex assay that permits the simultaneous amplification of target DNA of 13 high-risk and one possibly oncogenic human papillomaviruses (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68).

B set is a multiplex assay that permits the simultaneous amplification of target DNA of 5 possibly oncogenic (26, 61, 69, 73, 82) and 9 low-risk human papillomaviruses (6, 11, 40, 42, 43, 44, 53, 54, 70).

Operating mode:

1. Use 1mL of PBS-suspended anal swab specimen.

1. DNA isolation using DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany).

Preparation for Real-Time PCR:

5 mL 4X HPV28 A TOM or B TOM
5 mL 4X Anyplex PCR Master Mix (with UDG)
5 mL RNase-free Water
5 mL sample's nucleic acid
The total volume of PCR reaction is 20mL

1. The CFX96TM Real-time PCR System (Bio-Rad, Marnes-la-Coquette, France) amplification cycling program setup for the Anyplex TM II HPV 28 is pre-registered. The analysis of the results is realized using the Anyplex TM II HPV 28 software.