

# **HPV VACCINATION ON HPV-POSITIVE WOMEN: USING FUNCTIONAL IN-VITRO MODELS TO UNDERSTAND HPV INFECTIVITY AND TRANSMISSION**

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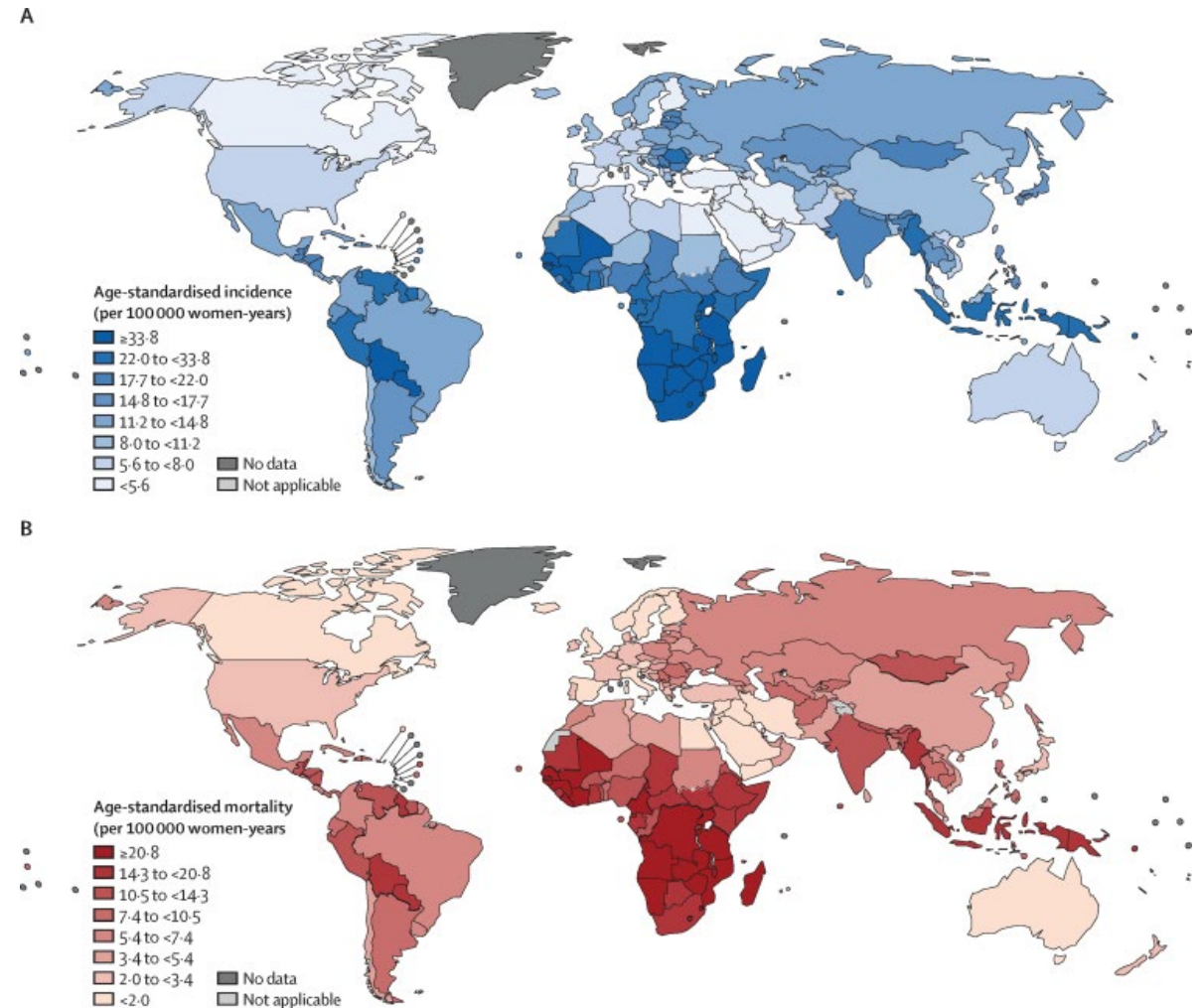
Institut d' Investigacions Biomèdiques de Bellvitge  
(IDIBELL)

# HUMAN PAPILLOMAVIRUS

Worldwide, more than 610,000 cancer cases are annually attributed to Human Papillomavirus (HPV).

HPV16 and HPV18 → Responsible for more than 70% cervical cancers.

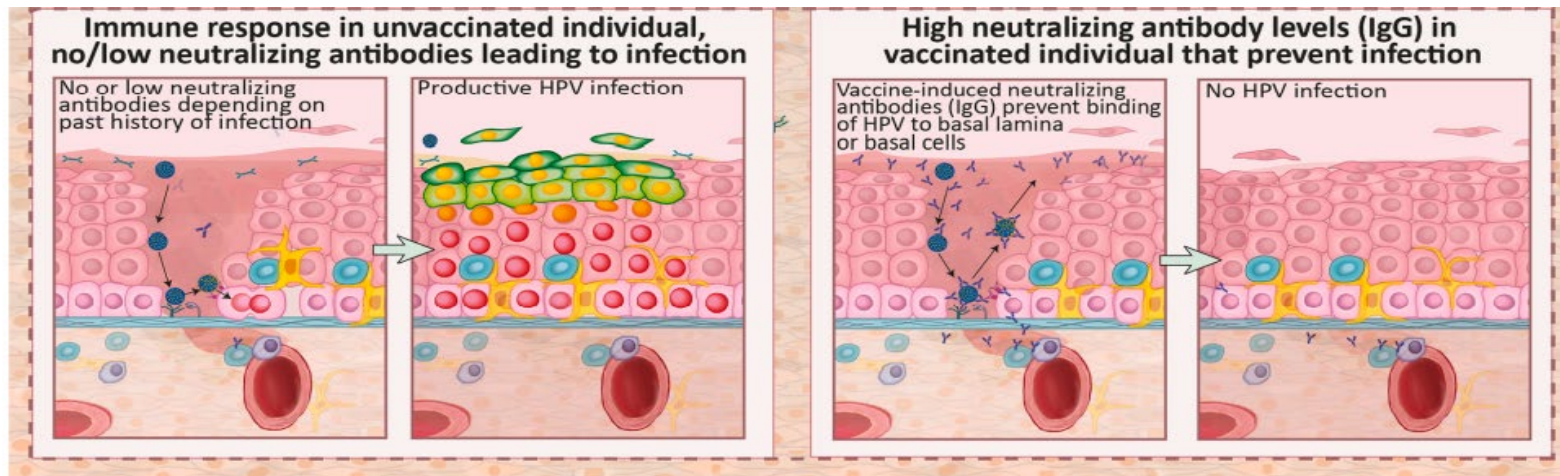
Sexually transmitted infection



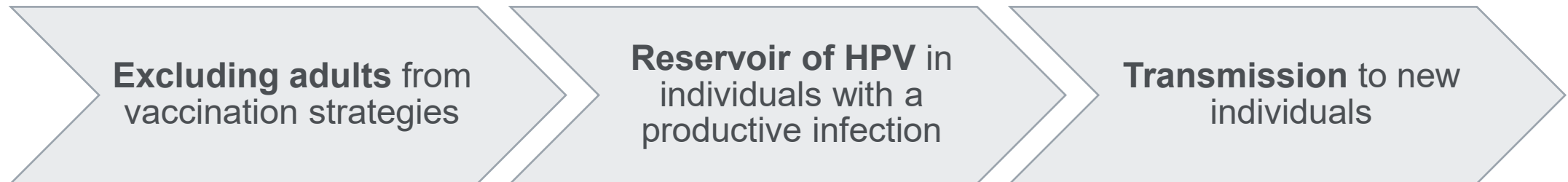
Global estimates of incidence (A) and mortality (B) of cervical cancer in 2020, **Singh et al., 2022**

# HPV vaccines

- **HPV vaccines based on VLPs** have demonstrated high safety, immunogenicity, and effectiveness for the prevention of infection and associated malignant lesions.
- **Neutralizing antibodies (nAbs)** are essential in preventing the HPV viral particles from attaching to the surface of epithelial cells, thereby inhibiting new infections.
- **nAbs** produced after vaccination provide much longer-lasting and more effective individual protection than those produced by natural infection

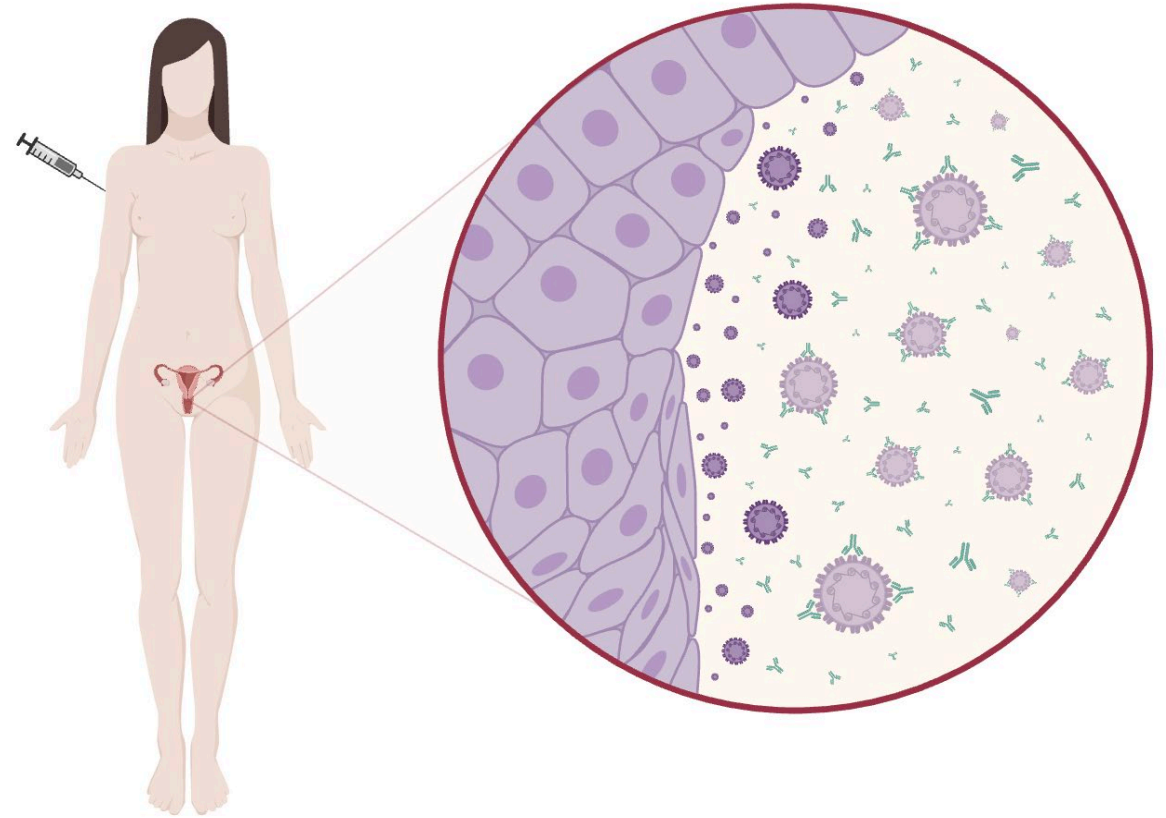


- **HPV prophylactic vaccination** has proven effective in preventing new infections, but it does not treat existing HPV infections or associated diseases.
- Therefore, **vaccination programs are mainly focused on young women.**



# Could HPV vaccination have an impact on HPV positive individuals reducing HPV transmission?

- HPV vaccination of HPV-positive women is equally immunogenic and completely safe
- nAbs are present in cervical, oral, anal and urine samples
- HPV virions are realized in the cervical mucosa
- Vaccination reduces the risk of clinical disease relapse after treatment
- vaccine-induced antibody responses are significantly higher than natural serological responses



**Vaccine induce nAbs that join to new HPV released particles and inhibit their infectivity?**



# VACCINATION IN HPV-POSITIVE INDIVIDUALS

> [JMIR Res Protoc](#). 2019 Jan 16;8(1):e11284. doi: 10.2196/11284.

## Human Papillomavirus Infection and Transmission Among Couples Through Heterosexual Activity (HITCH) Cohort Study: Protocol Describing Design, Methods, and Research Goals

Mariam El-Zein<sup>1</sup>, François Coutlée<sup>2</sup>, Pierre-Paul Tellier<sup>3</sup>, Michel Roger<sup>2</sup>, Eduardo L Franco<sup>1</sup>, Ann N Burchell<sup>1 4 5</sup>; HITCH Study Group

Randomized Controlled Trial > [Sex Transm Dis](#). 2022 Jun 1;49(6):414-422.

doi: 10.1097/OLQ.0000000000001620. Epub 2022 Mar 2.

## Protection to Self and to One's Sexual Partner After Human Papillomavirus Vaccination: Preliminary Analysis From the Transmission Reduction And Prevention with HPV Vaccination Study

Aaron MacCosham<sup>1</sup>, Mariam El-Zein<sup>1</sup>, Ann N Burchell, Pierre-Paul Tellier<sup>2</sup>, François Coutlée<sup>3</sup>, Eduardo L Franco<sup>1</sup>; TRAP-HPV study group

Observational Study > [PLoS One](#). 2019 Mar 4;14(3):e0212927.

doi: 10.1371/journal.pone.0212927. eCollection 2019.

## Effect of the bivalent HPV vaccine on viral load of vaccine and non-vaccine HPV types in incident clearing and persistent infections in young Dutch females

Pascal van der Weele<sup>1 2</sup>, Martijn Breeuwsma<sup>1</sup>, Robine Donken<sup>1 2</sup>, Elske van Logchem<sup>1</sup>, Naomi van Marm-Wattimena<sup>1</sup>, Hester de Melker<sup>1</sup>, Chris J L M Meijer<sup>2</sup>, Audrey J King<sup>1</sup>

Affiliations + expand

PMID: 30830913 PMCID: [PMC6398842](#) DOI: [10.1371/journal.pone.0212927](#)

Women that reported being vaccinated showed less HPV transmission to their partners and lower viral loads (for HPV6/11/16/18 infections), when compared to unvaccinated women.

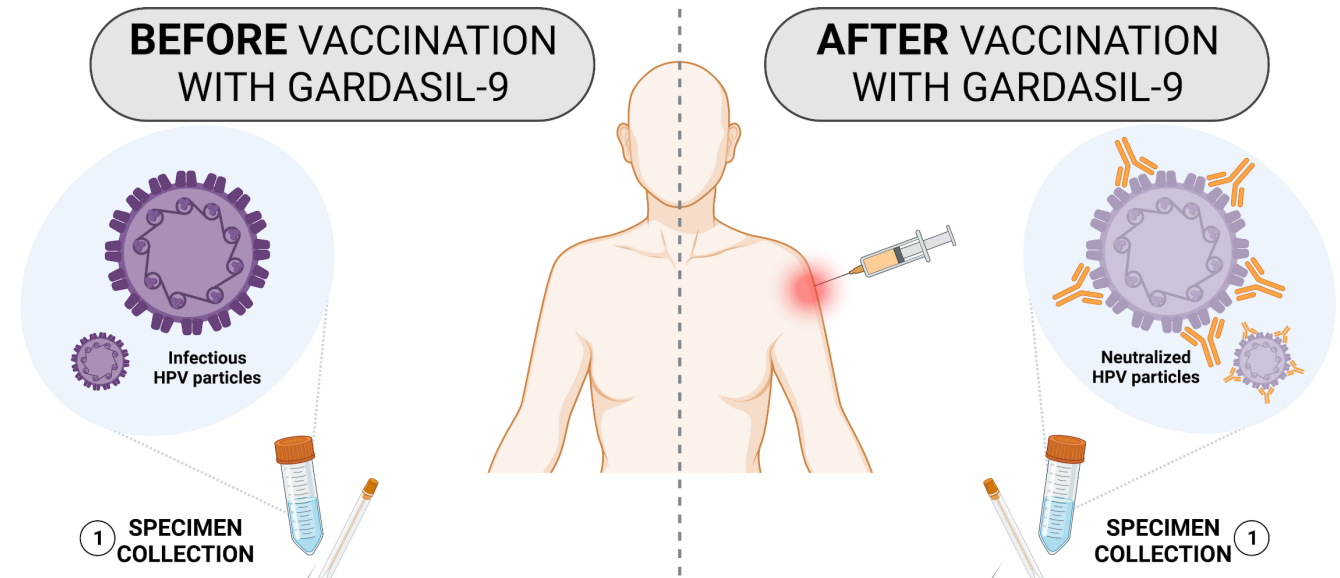
Inconclusive evidence regarding if HPV vaccination could reduce transmission and in turn protect sex partners from new vaccine-preventable infections.

Lower viral load in persistent infections

# RIFT

Reduction of Viral Infectivity and Transmission in HPV16/18 positive women before and after vaccination with nonavalent HPV vaccine

Evaluate if a 3-dose regimen of 9 valent HPV vaccine could **reduce the infective capacity** of body fluids from **HPV16/18-positive women** using a in vitro functional model to evaluate infectivity



## RIFT STUDY

This non-randomized, open-label trial, has been designed to recruit two different cohorts of **non-vaccinated adult women, positive for HPV16 and/or HPV18:**

- **RIFT-HPV1** (39 subjects): Women with a previous HPV16 and/or HPV18 positive cervical test and no apparent cervical lesions or cervical intraepithelial neoplasia (CIN) 1/2 lesions, eligible for conservative treatment.
- **RIFT-HPV2** (30 subjects): Women with a previous HPV16 and/or HPV18 positive anal test and no apparent anal lesions or anal lesions eligible for conservative treatment, as well as adult women with an HPV16 and/or HPV18 positive cervical test and HPV-associated vulvar lesions.



## Recruitment centre

- Gynaecology Unit, Bellvitge University Hospital (HUB), L'Hospitalet de Llobregat, Barcelona, Spain

## Satellite sites

- Department of Obstetrics and Gynaecology, Hospital del Mar – Mar Health Park, Barcelona, Spain
- Sexual and Reproductive Health Care Center – ASSIR, Delta del Llobregat, Barcelona, Spain
- HIV and STD Unit, Bellvitge University Hospital (HUB), Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain
- Cervical Cancer Screening Technical Office, Cancer Epidemiology Research Programme, Catalan Institute of Oncology, L'Hospitalet de Llobregat, Barcelona, Spain



<http://ico.gencat.cat>

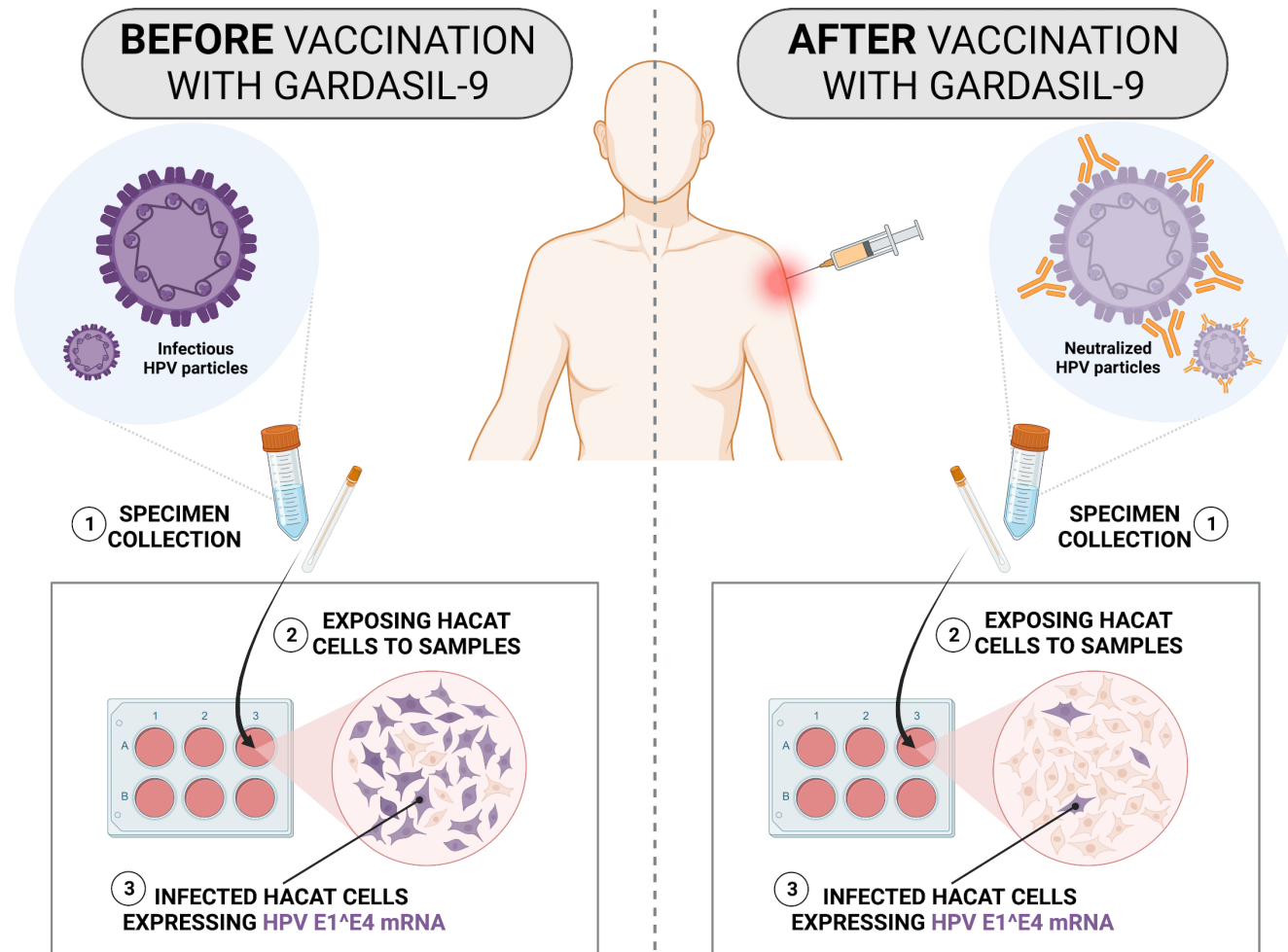
# STUDY PROCEDURES

- Questionnaire
- Sample Collection
  - Cervical
  - Vulvar
  - Anal
  - First-void Urine
  - Oral
  - Blood
- Vaccine administration

	STUDY PERIOD			
	Visit 1	Visit 2	Visit 3	Visit 4
TIMEPOINT	Day 1	Month 2 (±3 weeks)	Month 6 (±4 weeks)	Month 7 (-3/+7 weeks)
ENROLMENT				
Eligibility screen	X			
Informed consent	X			
INTERVENTIONS				
Urine hCG pregnancy test	X	X	X	X
Temperature measurement	X	X	X	X
Questionnaire	X	X	X	X
Height and weight measurement	X			
Medical history and prior/concomitant medication and vaccination	X	X	X	X
Gynaecological examination	X	X	X	X
Cervical sample collection	X	X	X	X
Vulvar sample collection	X	X	X	X
Anal sample collection	X	X	X	X
First-void urine collection	X	X	X	X
Oral sample collection	X	X	X	X
Blood sample collection	X	X	X	X
ASSESSMENTS				
Post-vaccination immediate AE <sup>1</sup>	X	X	X	
AE/SAE assessment		X	X	X

## PRIMARY ENDPOINT

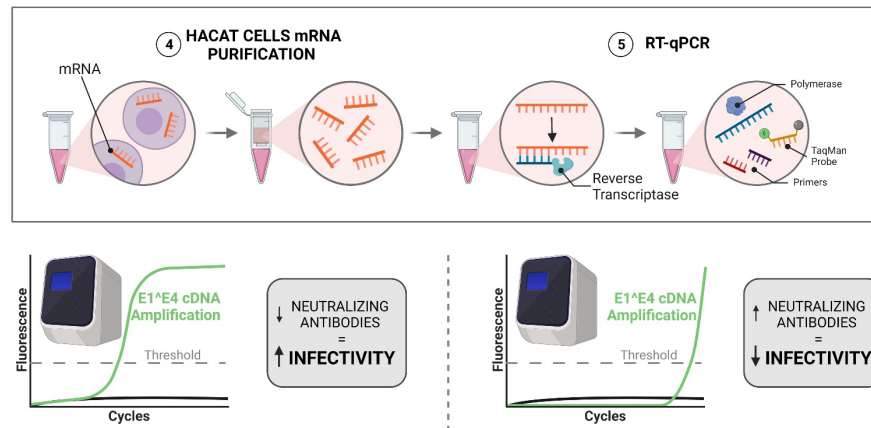
Measure HPV infectivity before and after vaccination through the **in vitro infection** of HaCaT cells and the subsequent **expression of the HPV E1^E4** spliced transcript.



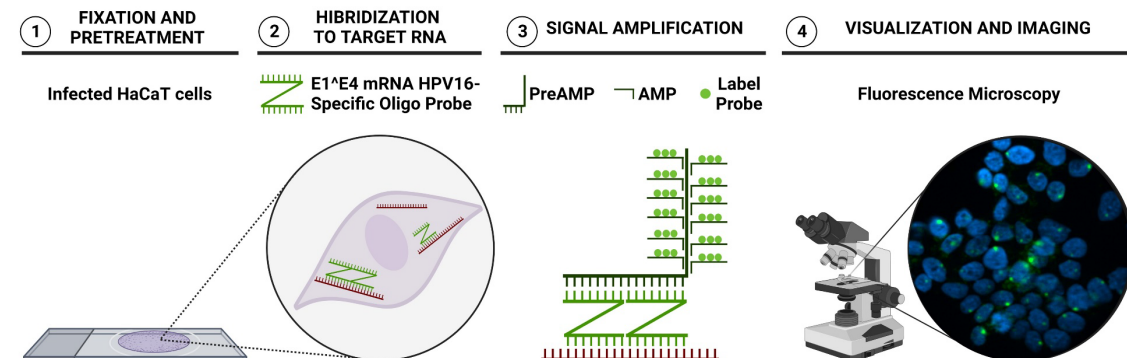
# In vitro evaluation of viral infectivity

Indirect quantification of infective HPV virions in a sample by quantifying HPV E1^E4 mRNA expression in HaCaT cells after incubation with the collected samples (cervical, anal and oral).

## RT-qPCR



## RNAscope Fluorescent Assay



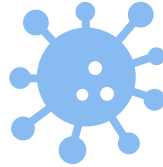
## COMPLEMENTARY ENDPOINTS



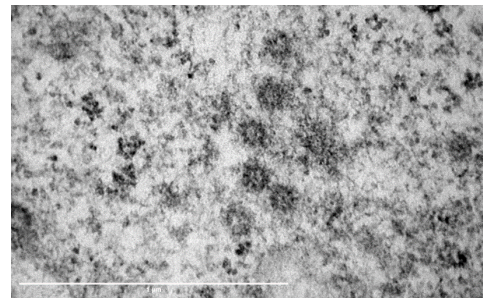
HPV DNA detection  
and genotyping



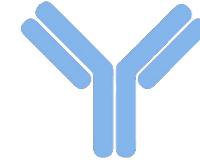
Anyplex / Allplex  
HPV28 Assay



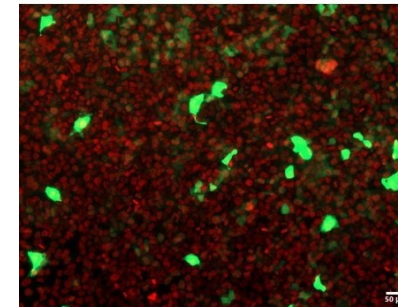
HPV16/18 virion  
detection



Anti-HPV L1 ELISA /  
Electron Microscopy



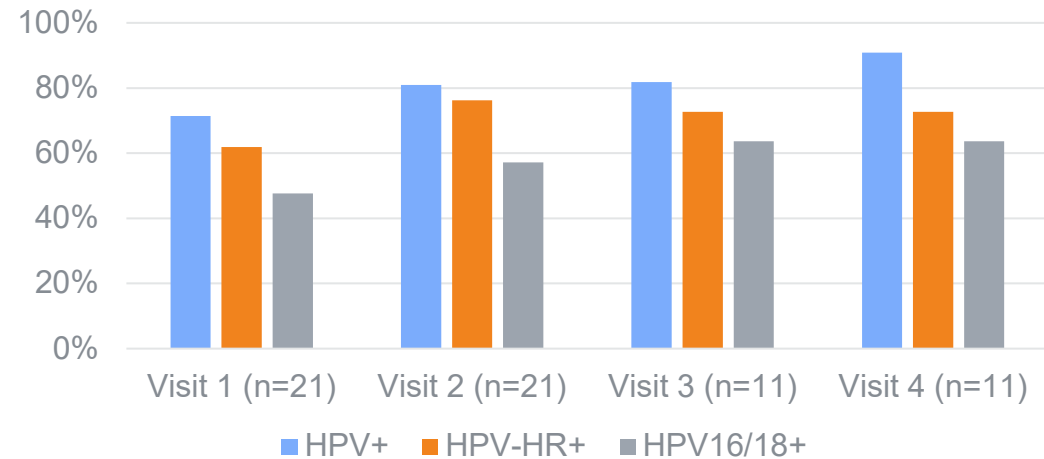
Anti-HPV L1 detection  
in body fluids



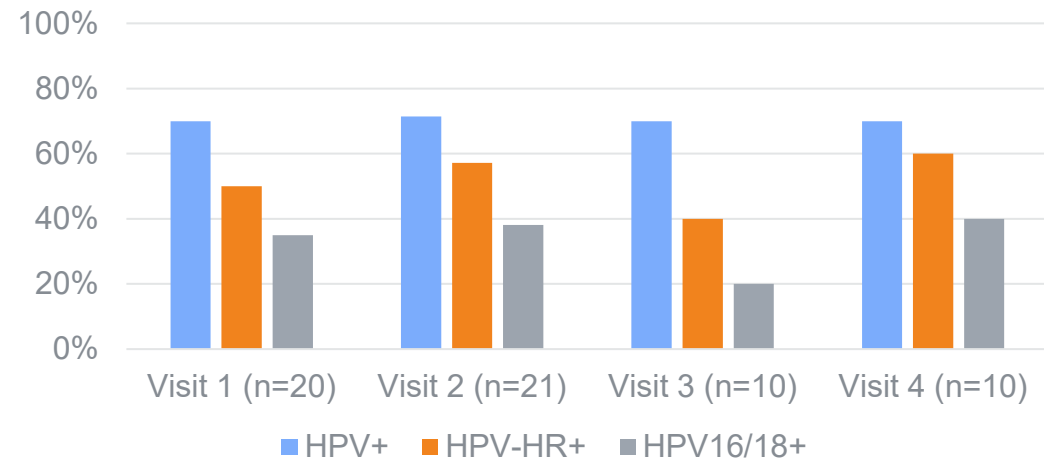
Anti-HPV L1 Ab ELISA /  
Neutralisation Assays

- 23 patients, 68 visits in total:
  - RIFT-HPV1: 21 patients
  - RIFT-HPV2: 2 patients
- 408 samples received and processed
- 136 DNA extractions and HPV detection and genotyping (cervical and anal samples)
  - 3 invalid results (anal samples)

### RIFT-HPV1 CERVICAL HPV POSITIVITY



### RIFT-HPV1 ANAL HPV POSITIVITY

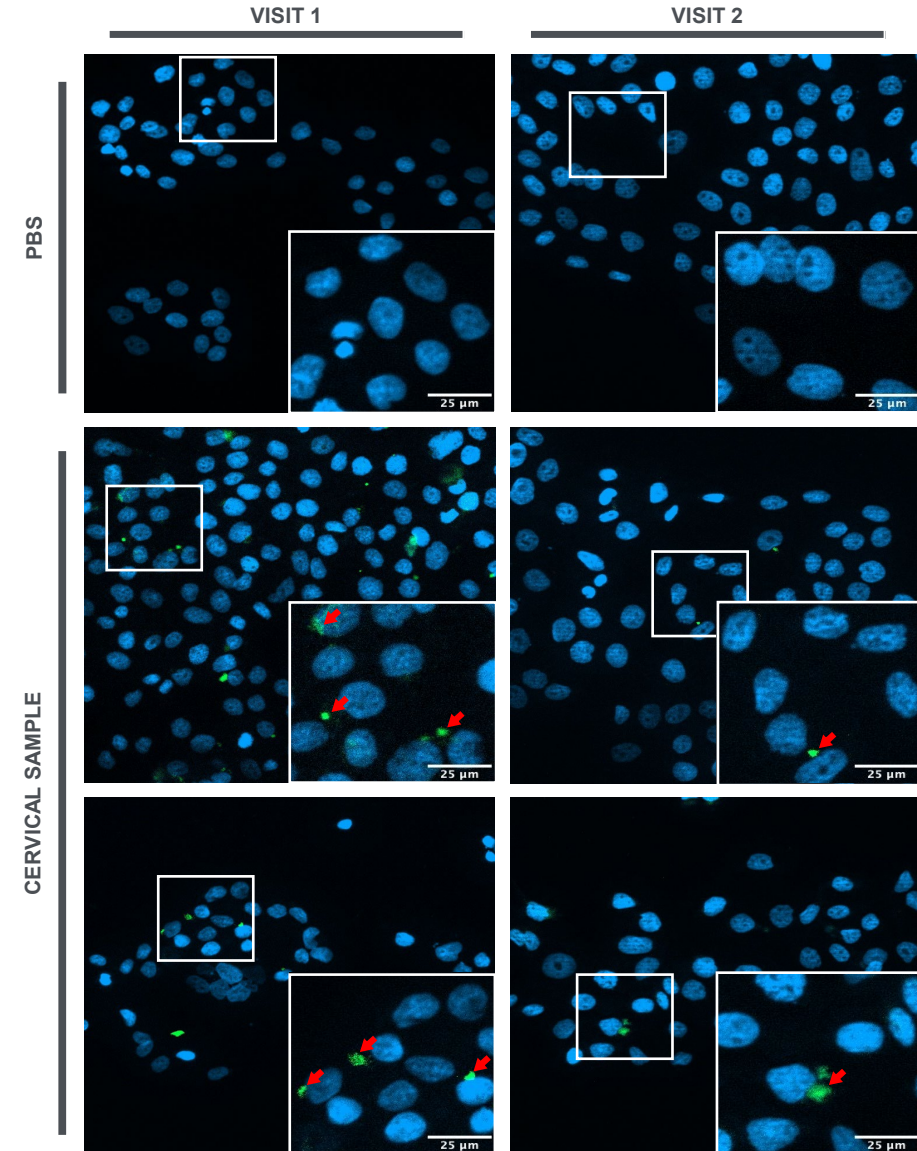




# INFECTIVITY ASSAYS: RNASCOPE

HaCaT cell cultures infected with cervical samples from a patient on visit 1 (before vaccination) and visit 2 (after the first dose of Gardasil-9).

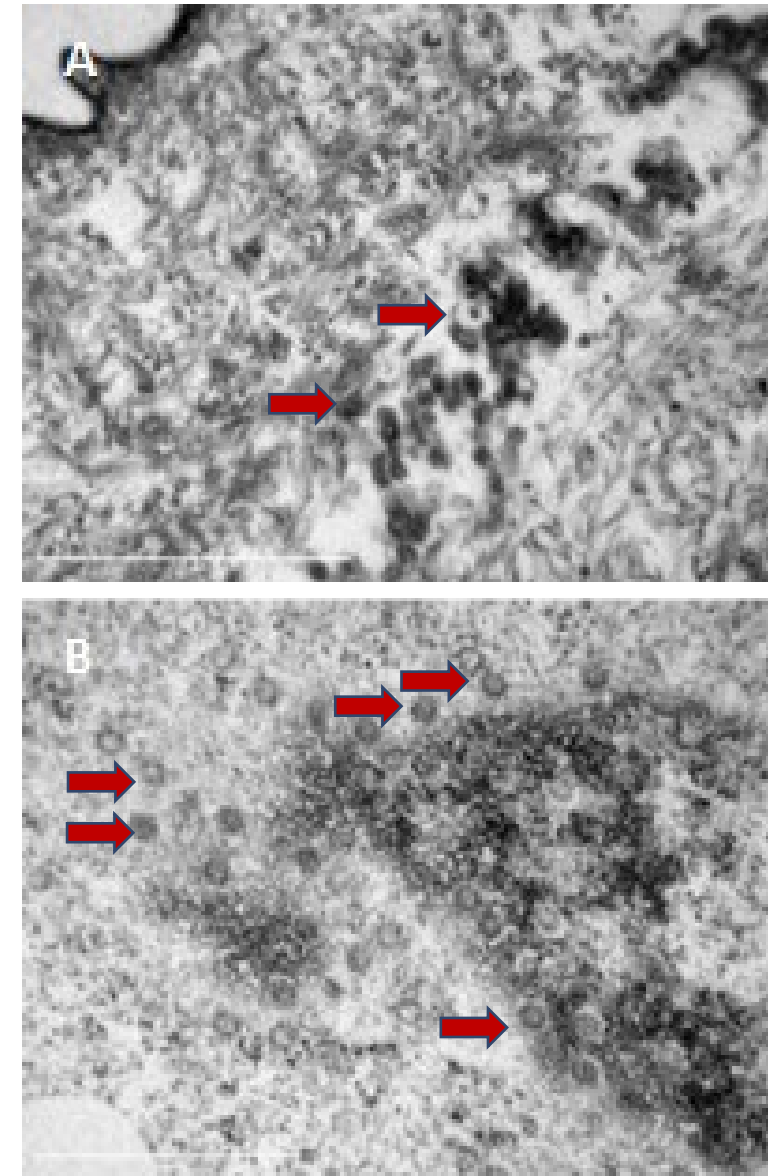
Current variations to the original protocol are being evaluated in order to optimise the assay and enable quantification of infectivity levels.



## VIRION DETECTION

Cervical sample cells from an HPV-positive subject (A) and PsV-infected cells (B) were visualized in the transmission electron microscope.

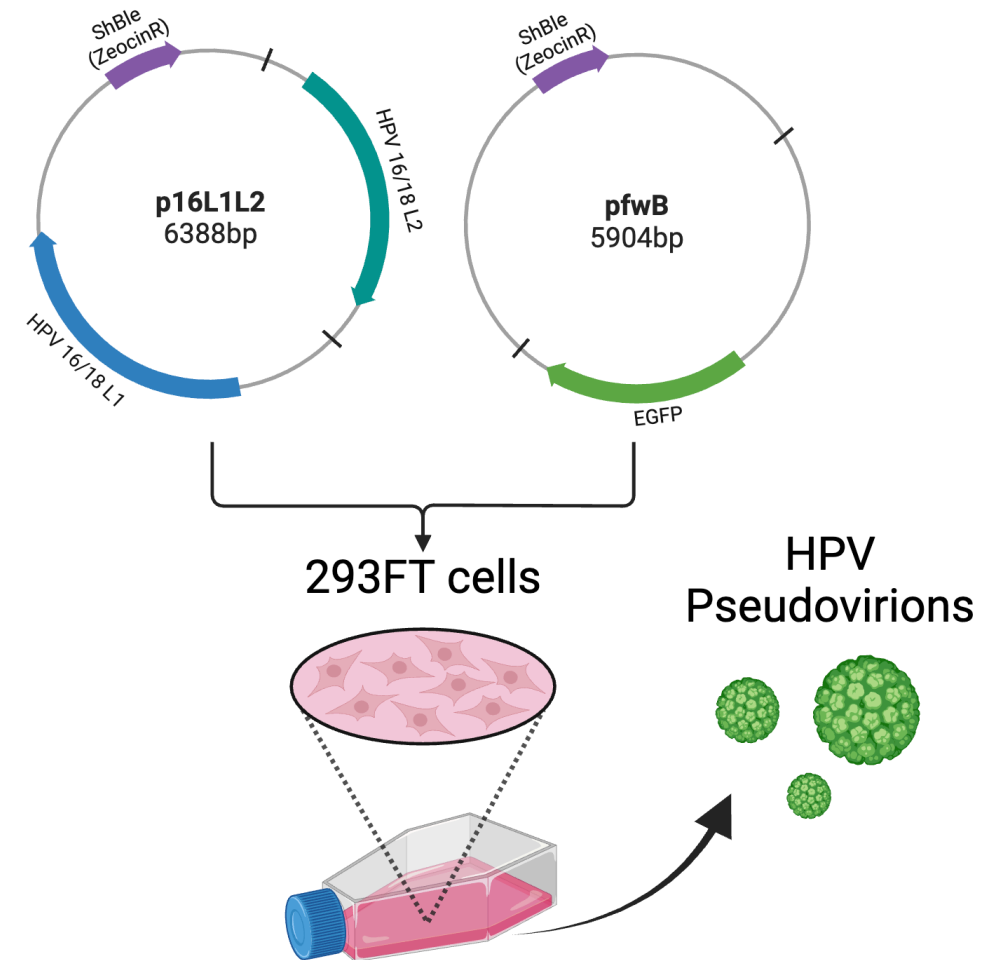
41 cervical samples were tested with a sandwich HPV16 L1 ELISA.



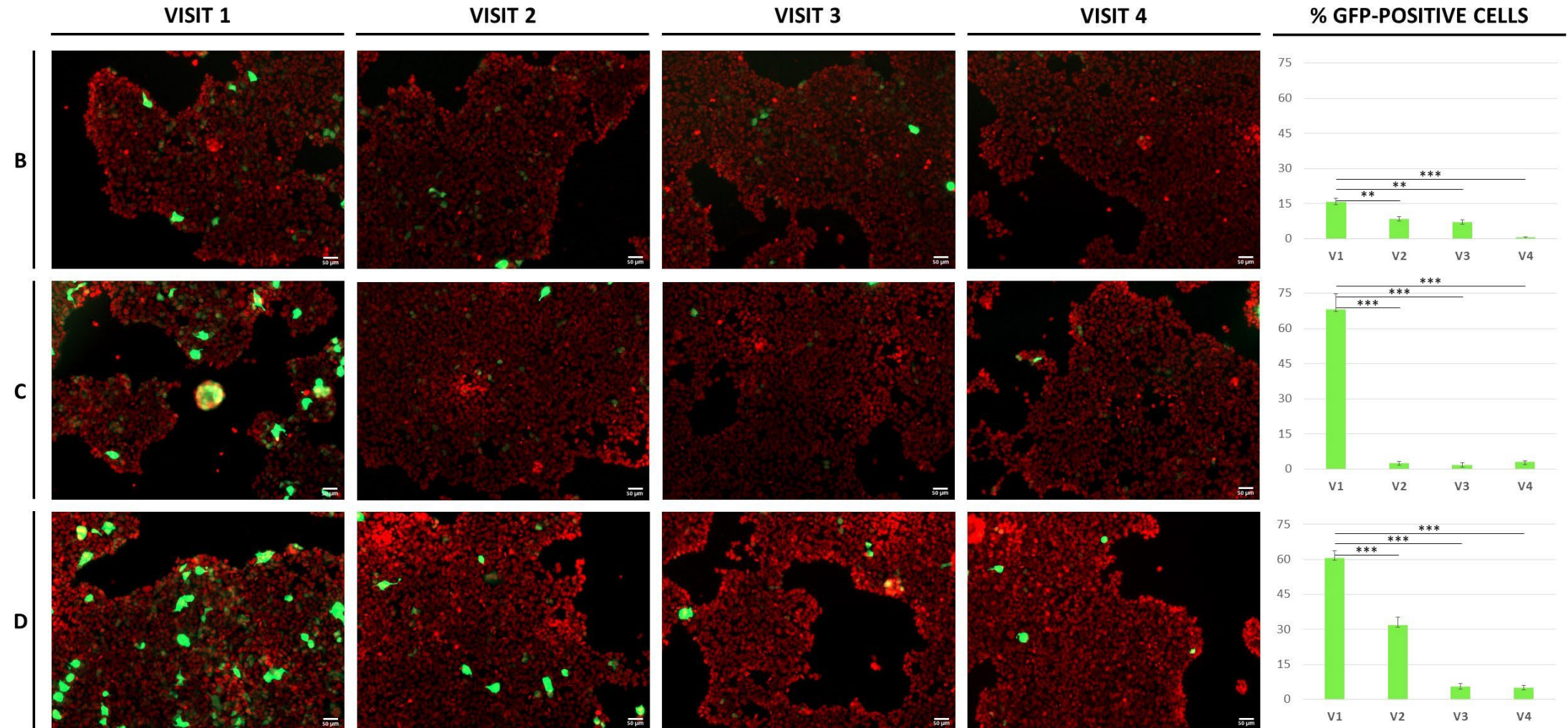
## NEUTRALISATION ASSAYS

HPV pseudovirions were generated by transfecting 293TT cells with an L1/L2 plasmid for HPV16 or HPV18, together with a GFP reporter plasmid.

Cervical samples and serum are tested for their ability to neutralize the pseudovirions in 293TT cultures, before and after vaccination.

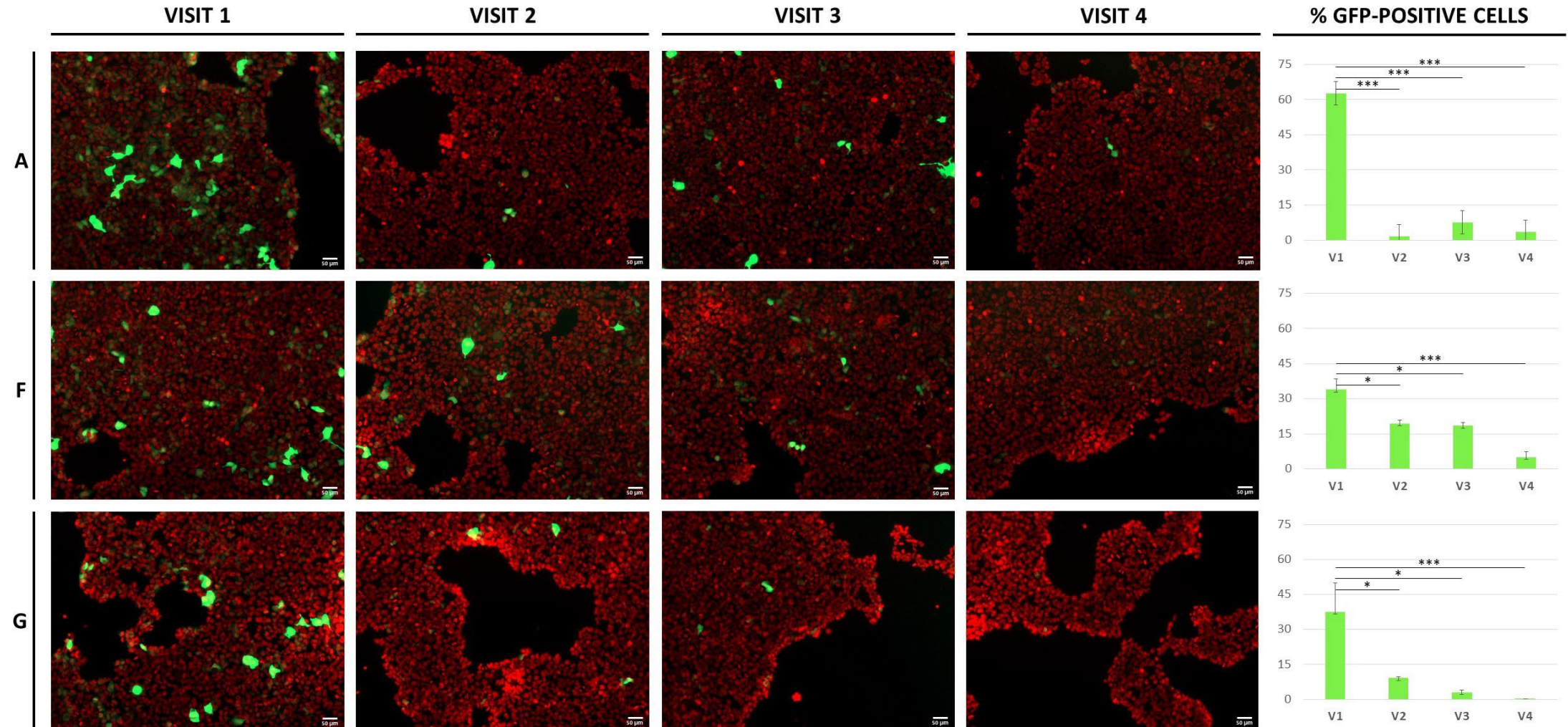


# NEUTRALISATION ASSAYS: Cervical Samples (Negative)

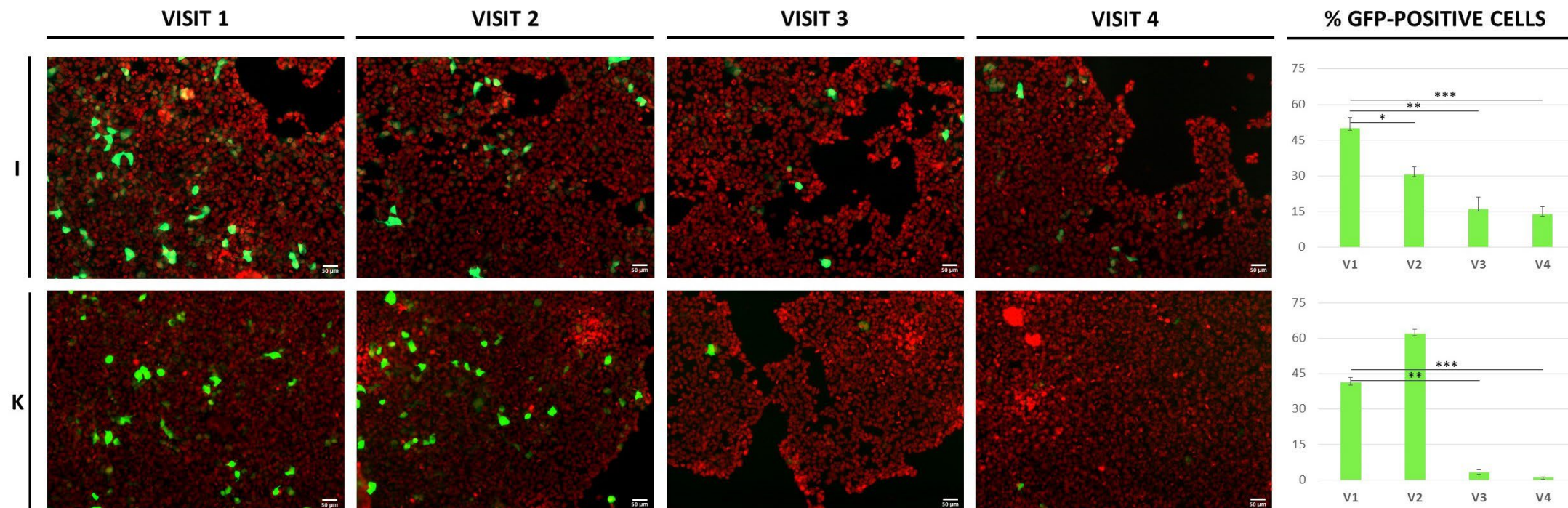




# NEUTRALISATION ASSAYS: Cervical Samples (Positive)



# NEUTRALISATION ASSAYS: Cervical Samples (Positive)





## NEUTRALISATION ASSAYS

- 75.8% of HPV16-pseudovirion neutralization after one dose (n=20). In those subjects that had completed the 3-dose schedule, 90.7% and 95.6% of neutralization were observed after two and three doses, respectively (n=9).
- Neutralizing effects were comparable between positive and negative subjects, showing a 77.9% and a 74.1% of neutralization after one dose, respectively.

\* Neutralisation assays in oral and anal samples are currently ongoing

## NEXT STEPS

- Finalize the recruitment (more than one recruitment sites)
- Infectivity Assays:
  - Try different cell types: HaCaT, HeLa and 293TT
  - Sample pellet lysis for virion release
- Virion detection:
  - Alternative assays for HPV L1 protein detection in samples
- Neutralisation Assays:
  - Comparing our GFP approach with the SEAP standard
  - Characterizing neutralising levels in all samples (cervical, oral and anal)

## PLOS ONE

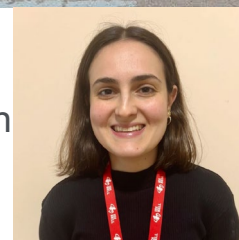
### STUDY PROTOCOL

# Assessing the reduction of viral infectivity in HPV16/18-positive women after one, two, and three doses of Gardasil-9 (RIFT): Study protocol

Victoria López-Codony<sup>1,2</sup>, Álvaro de Andrés-Pablo<sup>1,2</sup>, Angelica Ferrando-Díez<sup>3</sup>, Maria Eulàlia Fernández-Montoli<sup>4</sup>, Marta López-Querol<sup>1</sup>, Sara Tous<sup>1,5</sup>, Carlos Ortega-Expósito<sup>4</sup>, Juan Carlos Torrejón-Becerra<sup>4</sup>, Yolanda Pérez<sup>4</sup>, Anna Ferrer-Artola<sup>6</sup>, Josep Maria Sole-Sedeno<sup>7</sup>, Clara Grau<sup>8</sup>, Blas Rupérez<sup>8</sup>, Maria Saumoy<sup>9</sup>, Mónica Sánchez<sup>9</sup>, Paula Peremiquel-Trillas<sup>1,2,5</sup>, Laia Bruni<sup>1,5</sup>, Laia Alemany<sup>1,5</sup>, Francesc Xavier Bosch<sup>1,5,10†</sup>, Miquel Angel Pavón<sup>1,5†\*</sup>



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Alvaro de Andres