

Human papillomaviruses (HPV) are the causative agents for the great majority of anogenital and oropharyngeal cancers as well as anogenital warts. Vaccination against Human Papillomaviruses (HPV) with virus-like particles (VLPs) consisting vaccines is recommended in Germany for children from nine to fourteen years. The aim of this work was to evaluate mechanisms of the T- and B-cell mediated immunity and a putative cross-reactivity against L1-VLPs of the HPV-Types 6, 11, 16 and 18 by fluorescent Enzyme-Linked-ImmunoSpot (EliSpot) Assay.

To evaluate T-cell mediated immunity, IFN γ , IL-2 and IL-5 were simultaneously detected in one well. Our hypothesis says, that fully vaccinated donors should express the most IFN γ , whereas partly vaccinated donors should express less IFN γ when stimulated with VLPs, in contrast to almost no IFN γ expression in non-vaccinated donors. The individual T-cell reaction has been monitored to elucidate dynamic changes in T-cell patterns. Therefore, IFN- γ (active response), IL-2 (memory response) and IL-5 (transient secretion during vaccination regime) were detected (Fig. 1). HPV specific B-cells were detected using fluorescently labeled VLPs from HPV types 6, 11, 16 and 18 to investigate potential cross-reactivity (Fig. 2).

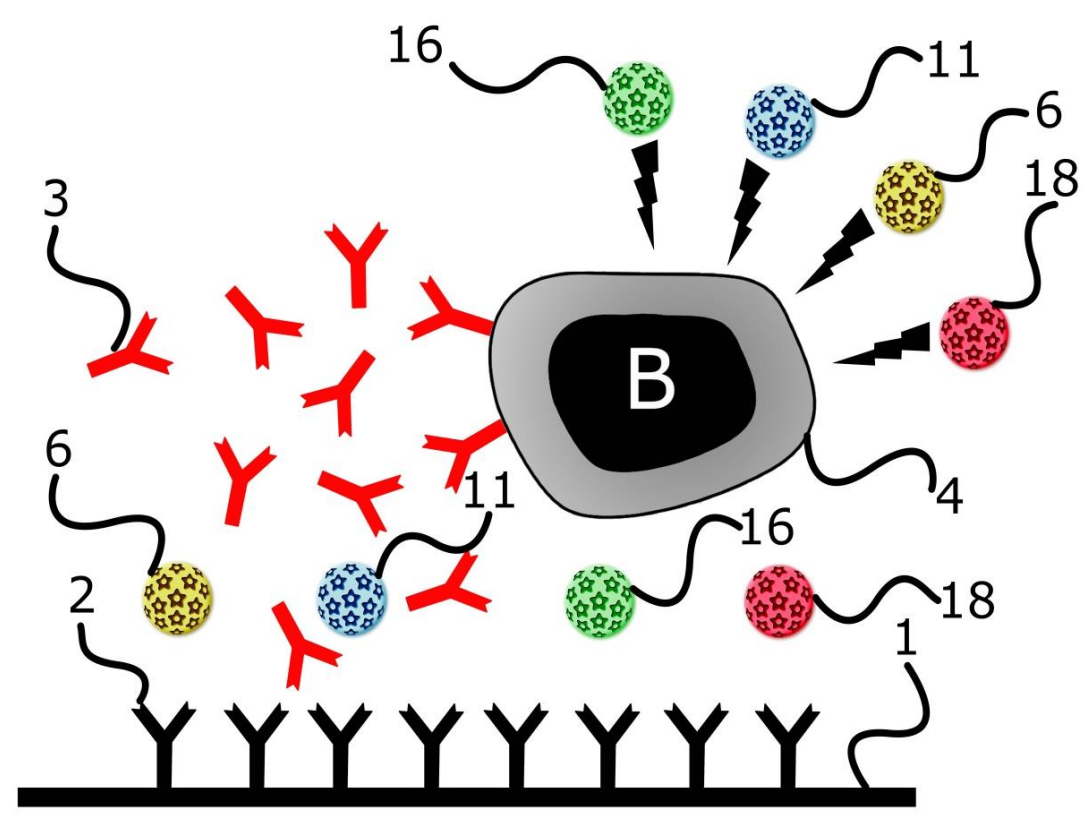
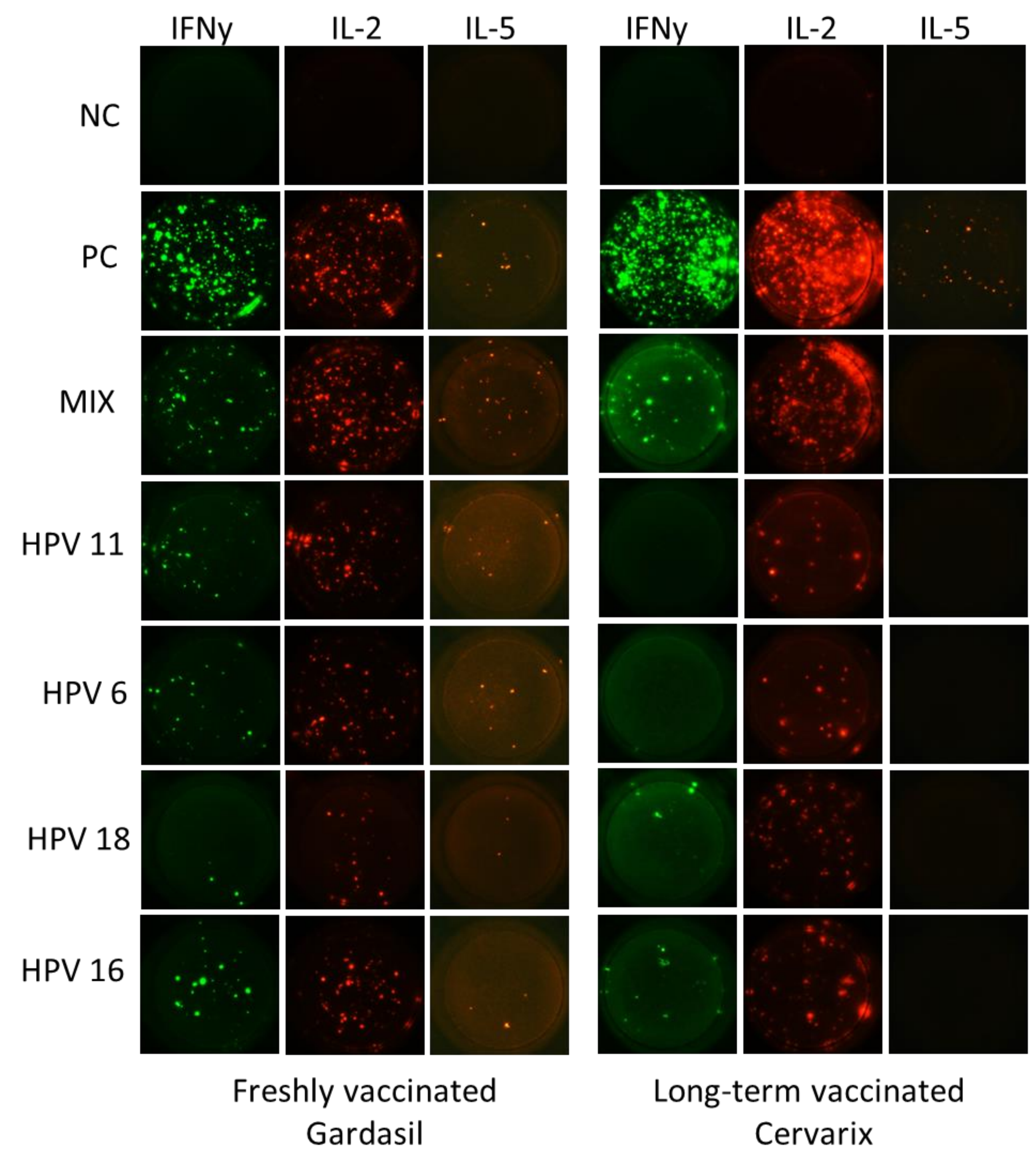


Fig.2: Schematic drawing of the used AID B-Cell EliSpot. Numbers indicate the following: 1- plate with PVDF membrane for fluorescent applications; 2- coated antibodies; 3- secreted anti-HPV antibodies from B-cells; 4- antibody secreting B-cell. 6- HPV6 VLPs, 11- HPV11 VLPs, 16- HPV16 VLPs and 18- HPV18 VLPs. B-cells were incubated o/n with fluorescently labeled VLPs. Secreted antibodies from donor bind to VLPs and are captured by the coated antibodies on the plate.

Fig. 1: Representative donors freshly vaccinated with Gardasil (left) and long-term vaccinated with Cervarix (right) from 3-colour T-cell EliSpot. Cells were stimulated with VLPs from HPV 6, 11, 16, 18 and a mixture of all (mix) at 3 μ g/ml.

NC = negative control cells only, PC = positive control, cells stimulated with Pokeweed Mitogen. Shown here are the individual fluorescence channels: green- FITC-IFN γ (left row), red-Cy3-IL-2 (middle row) and red-Cy5- IL-5 (right row). Pictures were obtained with an AID GmbH iSpot Spectrum Reader.



For HPV- reactive T-cells, this was proven with EliSpot assays and the groups were clearly distinguishable. Also, fully vaccinated donors showed the highest IL-2 expression. IL-5 expression was only detectable in vaccinated donors, which indicates functional T helper 2 T-cells against VLPs from HPV 11, 16 and 18 (Fig. 3). For evaluating functional B-cells, and cross-protection potential of the different vaccinations, VLPs from HPV 6&11 (low-risk-mix) and 16&18 (high-risk-mix) were labeled with different fluorescent dyes to evaluate B-cell numbers for both mixes in one well in parallel (Fig. 4). We were able to detect a dose-dependent spot number for all HPV types in vaccinated donors which were for all types higher than for non-vaccinated donors. B-cell reactions to the low-risk pool from patients exclusively vaccinated with high-risk strains could be observed. This indicates a cross-protective cellular immune reaction after vaccination.

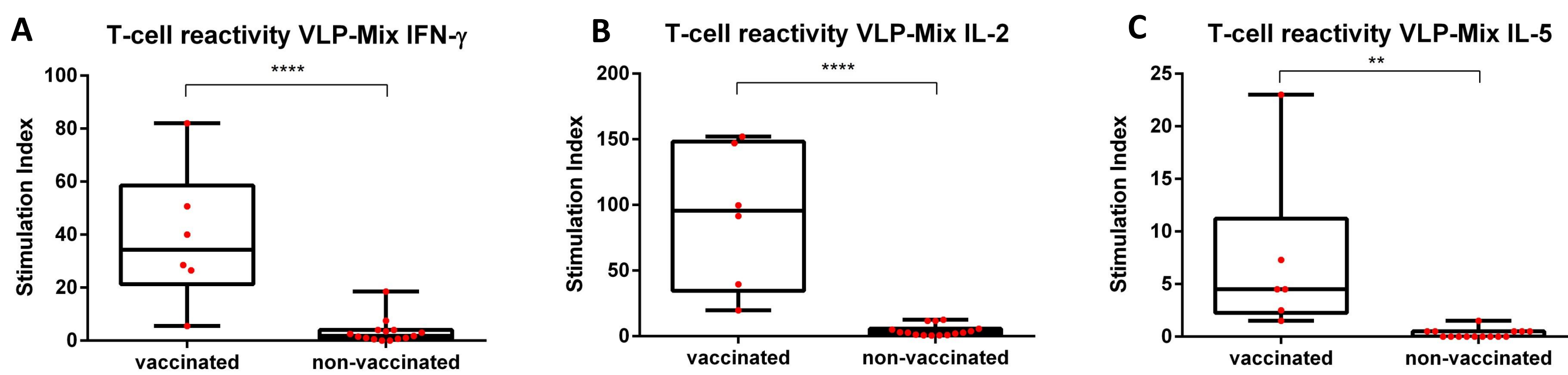


Fig. 3: T-cell reactivity for IFN γ after VLP-Mix (HPV 6, 11, 16, 18) stimulation. IFN γ (A), IL-2 (B) and IL-5 (C) were detected in parallel in one well. For vaccinated donors (n=6), highly significant (IFN- γ , IL-2) or significant (IL-5) T-cell reactions were detected, which was not the case in unvaccinated donors (n=15).

The range for effective vaccinations should be confirmed with more and well characterized donors. Furthermore, these trials could facilitate the detection of significant differences between vaccinated and non-vaccinated donors and open the possibility to define effectiveness of vaccination for cellular immune responses.

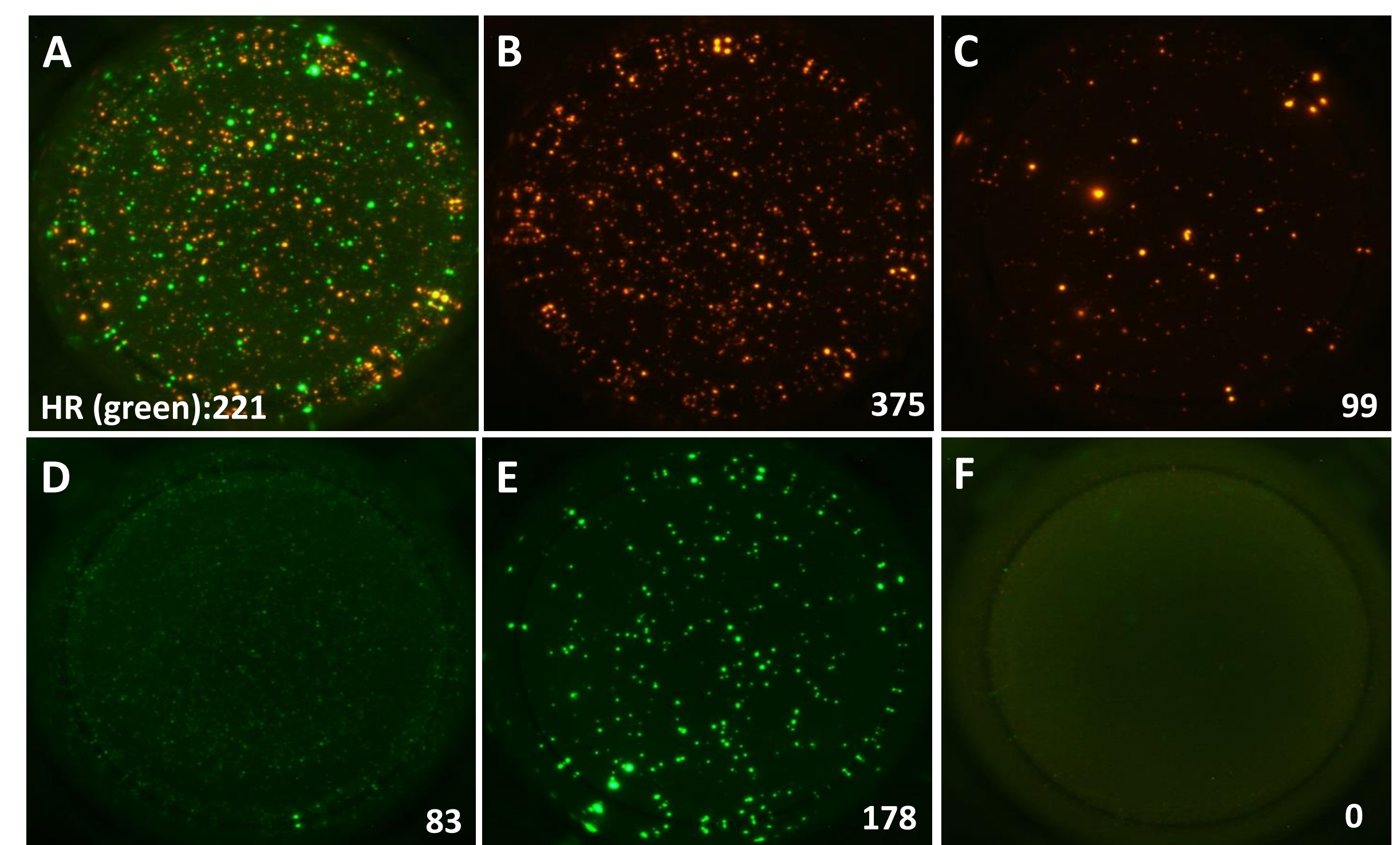


Fig 4: B-cell EliSpot of a vaccinated donor (3x Gardasil, last boost 2007) with fluorescently labelled HPV-VLPs. The particular figures successively represent the detected HPV-specific IgGs stimulated with VLP-Mix (A), HPV-11 (B), HPV-6 (C), HPV-18 (D), HPV-16 (E) and the negative control (VLP-mix in media Without PBMCs) (F). The numbers indicate the spot counts and are obtained with the AID GmbH iSpot Spectrum Reader.

