# Luminex®

## The Development of HPV Vaccines and the Contributions of Luminex xMAP® Technology

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## Introduction

Human papillomavirus (HPV) is the most common sexually transmitted infection, with an incidence rate of over 14 million people each year.[1] Of the more than 100 known types of HPV, many are associated with illness in both men and women worldwide, and 13 types are described as being related to cancer by the International Agency for Research on Cancer.[2-3] In addition to cervical cancer, HPV has also been associated with diseases like cervical intraepithelial neoplasia, genital condyloma, laryngeal papillomatosis, and cancers of the penis, anus, and oropharynx.[3-4]

There are currently three globally licensed vaccines that protect against various types of HPV. The bivalent (2vHPV) vaccine, Cervarix® (**GlaxoSmithKline**), protects against HPV types 16 and 18, which are responsible for around 90% of cases of cervical cancer.[4] Gardasil® (**Merck & Co**), a quadrivalent (4vHPV) vaccine, protects against HPV types 16 and 18, as well as genital warts caused by HPV types 6 and 11.[5] Finally, Gardasil® 9 (Merck & Co), a 9-valent (9vHPV) vaccine, also protects against HPV types 31, 33, 45, 52, and 58, providing protection against most cervical cancers and genital warts, along with HPV-related vulvar, vaginal, and anal cancers.[5-8]

The development and clinical testing of multivalent vaccines has traditionally required running separate tests to measure type-specific antibodies to different HPV genotypes.[9-12] The Gardasil® 4vHPV and 9vHPV vaccines in particular protect against multiple types of HPV,[5-8] and their development was optimized using Luminex's xMAP® multiplex technology. xMAP multiplexing technology enabled the measurement of type-specific antibodies to several HPV genotypes simultaneously.[9-12] This technology has powered the development of the initial vaccines from virus-like particles (VLPs), facilitated clinical trials, assisted in immunobridging studies, and provided insight into the vaccines' long-term effects through immunogenicity and epidemiological studies.

**Figure 1**. *Timeline of HPV vaccine development.*[15, 20, 58]
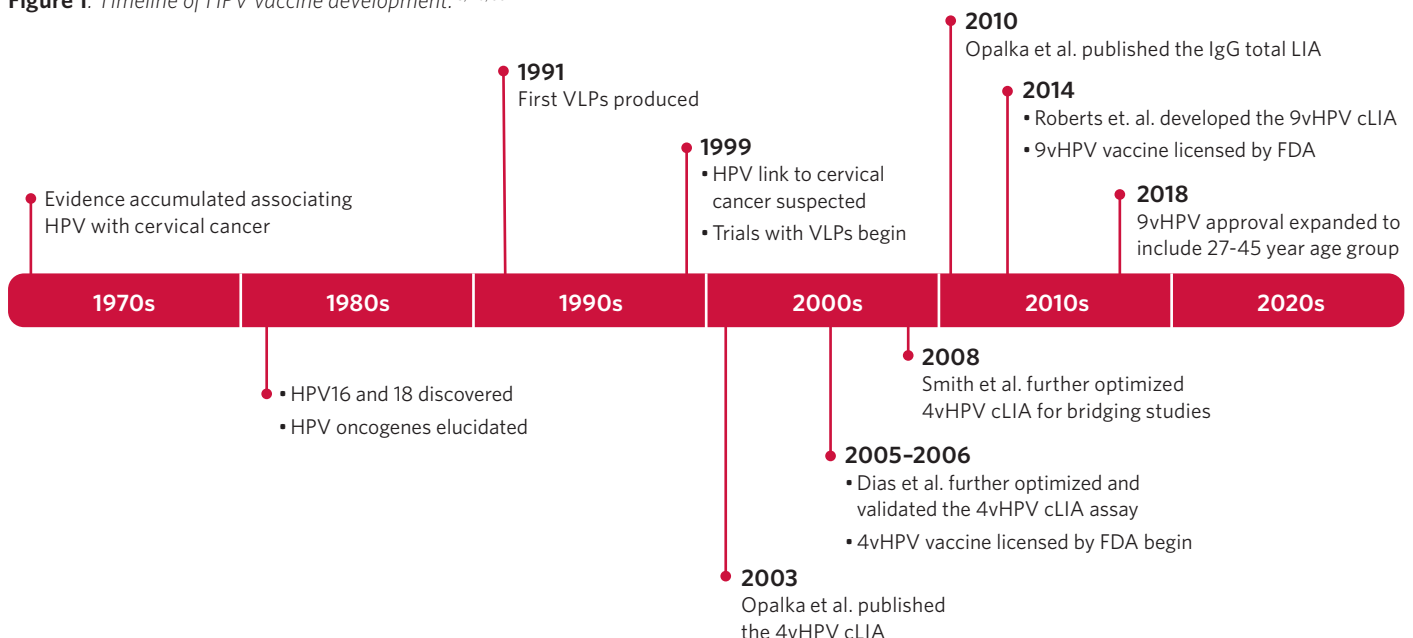
# Development of the Gardasil® 4vHPV vaccine using xMAP multiplexing assays

Opalka et al. first developed a **4vHPV competitive xMAP immunoassay (4vHPV cLIA)** to measure type-specific antibodies to several HPV genotypes simultaneously.[9] The group utilized Luminex xMAP Technology to enable the quantification of HPV type-specific antibody titers from a single serum sample. To accomplish this, HPV VLPs were covalently coupled to xMAP microspheres and added to 96-well plates. The competitive assay enabled the measurement of antibodies specific to HPV types 6, 11, 16, and 18 within serum, which prevented the binding of fluorescent HPV-specific antibodies labeled with phycoerythrin (PE).[10]

xMAP Technology offered a robust and sensitive system that was further optimized and validated for use in additional epidemiological studies and vaccine clinical trials.[9-10] Dias et al. worked to increase the clinical specificity and analytical sensitivity of the assay to help differentiate low-titer antibody responses of those infected with HPV from non-infected individuals. The authors also improved antibody specificity, optimized VLP and antibody concentrations, fine-tuned various components of the assay, and automated it using a TECAN Genesis Workstation.[10] The resulting high-throughput assay was sensitive and robust, and was used to monitor the immune response in Phase IIB and III clinical trials, as well as in later studies, where sensitivity and throughput were further optimized.[10, 13]

Despite its many advantages, the 4vHPV cLIA assay only measures a subset of the total immune response to vaccination. Although understanding the impact of the vaccine on induction of anti-HPV 6, 11, 16, and 18 type-specific neutralizing antibodies is essential, it is also critical to understand a more general measurement of the humoral immune response to the vaccine. **The total IgG xMAP immunoassay (total IgG LIA)** was consequently developed in-house to provide a supportive analysis tool throughout vaccine development and later studies.[11, 14] The assay utilizes VLPs coupled to nine distinct xMAP microspheres to detect IgG antibodies in serum to HPV type 6, 11, 16, 18, 31, 33, 45, 52, and 58 VLPs. Although the assay shares some components with the 4vHPV cLIA, it has key differences. Rather than restricting the measurement of binding to a single neutralizing epitope, the assay enables measurement of IgG antibodies anywhere on the complete VLP.[11, 14] When both the total IgG LIA and 4vHPV cLIA were used side by side to measure the antibody responses of young women to the 4vHPV vaccine across 48 months in a clinical study, the 4vHPV vaccine was shown to induce seroconversion in almost all participants. When these assays are used in combination, they provide a more complete understanding of the immune response to vaccination and have enabled the development, approval, and post-licensure studies of the Gardasil® 4vHPV vaccine.[14]

The international, randomized, double-blind, placebo-controlled Phase III FUTURE 1 (NCT00092521) and FUTURE 2 (NCT00092534) trials examined the prophylactic efficacy of the 4vHPV Gardasil® vaccine on cervical intraepithelial neoplasia, adenocarcinoma in situ, cervical cancer, condyloma acuminata, vulval intraepithelial neoplasia, vaginal intraepithelial neoplasia, vulvar cancer, and vaginal cancer.[15-18] The studies monitored incidence of disease in people that received the vaccine compared to a placebo group, and xMAP Technology was critical throughout the clinical trial process and in extension studies, where antibody responses to the vaccine were measured and data from these studies was often included as a secondary outcome clinical trials.[15-18] The international FUTURE 1 study examined 5,455 women between the ages of 16 and 24 who were randomized to either the 4vHPV vaccine or placebo group. The vaccine was shown to be effective at preventing anogenital diseases associated with HPV.[16] Similarly, the FUTURE 2 trial randomized over 12,000 women between the ages of 16 and 26 to receive 3 doses of either the 4vHPV vaccine or a placebo. Antibody titers from the 4vHPV cLIA assay were included as a secondary outcome. Women who received the 4vHPV vaccine had lower incidence of HPV-associated diseases compared with the placebo group.[17] In both of these studies and associated research, vaccination was repeatedly found to be effective at preventing lesions and disease associated with HPV infection compared to the placebo group, and xMAP Technology demonstrated that the vaccine showed hallmarks of a sustained antibody response.[16-19] The xMAP assays directly supported the development and clinical testing of this vaccine, and it was approved for use in women between the ages of 16 and 26 by the FDA in 2006.[20]

HPV infection can lead to disease in men as well, including anogenital condyloma acuminata, along with cancers of the penis, anus, and oropharynx.[21] An initial randomized, placebo-controlled, double-blind study examined the 4vHPV vaccine in 4,065 boys and men 16 to 26 years of age. The study aimed to elucidate whether the 4vHPV vaccine could reduce the incidence of HPV-related genital lesions. Antibody titers were monitored as a secondary outcome and it was found that the 4vHPV vaccine prevented infection with HPV types 6, 11, 16, and 18, along with incidence of genital leisons.[22] This trial was followed by a study examining the immunogenicity and safety of the vaccine in 150 men aged 27 to 45 years. xMAP Technology again helped measure antibody responses as a secondary outcome and found them comparable to vaccination results initially seen in the younger age group.[23]

## Bridging studies

In addition to being used for initial vaccine development and key clinical trials, the cLIA and total IgG LIA were used generate data supporting regulatory approval for additional populations that can be more difficult to study. These "bridging studies" examined several groups, including pre-adolescent boys and girls, mid-adult women, and special populations.[24]

The cLIA and total IgG LIA were immensely valuable in bridging studies examining the vaccine in pre-adolescent boys and girls since efficacy studies were not feasible in these populations.[25-26] The study examined girls (n= 506) and boys (n= 510) between 10 and 15 years of age, in addition to young women (n= 513) between 16 and 23 years of age. By month 7, antibody responses in the younger boys and girls were non-inferior and higher than young women in the 16- to 23-year age group. Seroconversion was further observed to be ≥99% for all HPV types in trial participants. Another study compared the immune response of sexually naïve boys versus girls between 9 and 15 years of age. Antibody responses of boys measured by the 4vHPV cLIA and total IgG assays were non-inferior to girls and demonstrated sustained protection.[25, 27]

xMAP Technology continued to be used to examine the 4vHPV Gardasil® vaccine in mid-adult women aged 25 to 45 years.[28-29] A total of 3,819 women with no recent history of genital warts or cervical disease received 3 doses of either the 4vHPV vaccine or a placebo. The previously described xMAP assays helped periodically measure antibody responses and were included as a secondary outcome. Similar to results seen in pre-adolescent boys and girls, mid adult-women had peak antibody titers that were non-inferior to those in the original 16- to 26-year group.[28-29]

The aforementioned xMAP assays have also been utilized to understand responses in special populations, such as people with HIV, rheumatic diseases, or in immunocompromised populations. For example, people with HIV have been shown to have an increased risk of infection with the types of HPV associated with oncogenic and non-oncogenic disease. Consequently, vaccination of this at-risk population may be especially important.[24, 30-31] Understanding HPV serology is important to get a fuller picture of both past and present HPV infections in at-risk populations, and to better understand a person's immune status before and after vaccination.[14-15] Toward this goal, a multiplex serology assay utilizing pseudovirions derived from mammalian cells was developed and validated. This **Pseudovirion-xMAP assay** utilizes

multiplexing technology to simultaneously measure antibody responses to 21 types of HPV.[31] The assay was used to understand the antibody responses to vaccination with either the 4vHPV Gardasil® or the bivalent Cervarix® vaccine in 91 HIV-infected people. The study found that vaccination of these HIV-infected people induced a neutralizing antibody response against the HPV types used in the vaccine, as well as other types of HPV not in the vaccine.[24, 32-33]

Vaccination in those with rheumatic diseases is similarly critical. People with rheumatic diseases may be at higher risk for persistent HPV infections and downstream disease, including pre-malignant and malignant leisons.[34] Consequently, a wide variety of studies have directly utilized the previously described xMAP multiplex assays to better understand the safety and immunogenicity of HPV vaccination in various high-risk populations with rheumatic diseases, including adult-onset systemic lupus erythematosus,[35-38] childhood-onset systemic lupus erythematosus, juvenile dermatomyositis,[35] and inflammatory bowel disease.[39] The assays overall showed that vaccination of these high-risk groups is safe and immunogenic, frequently leading to updated guidelines and recommendations in high-risk people.[35-39]

**Figure 2.** *The cLIA process.*
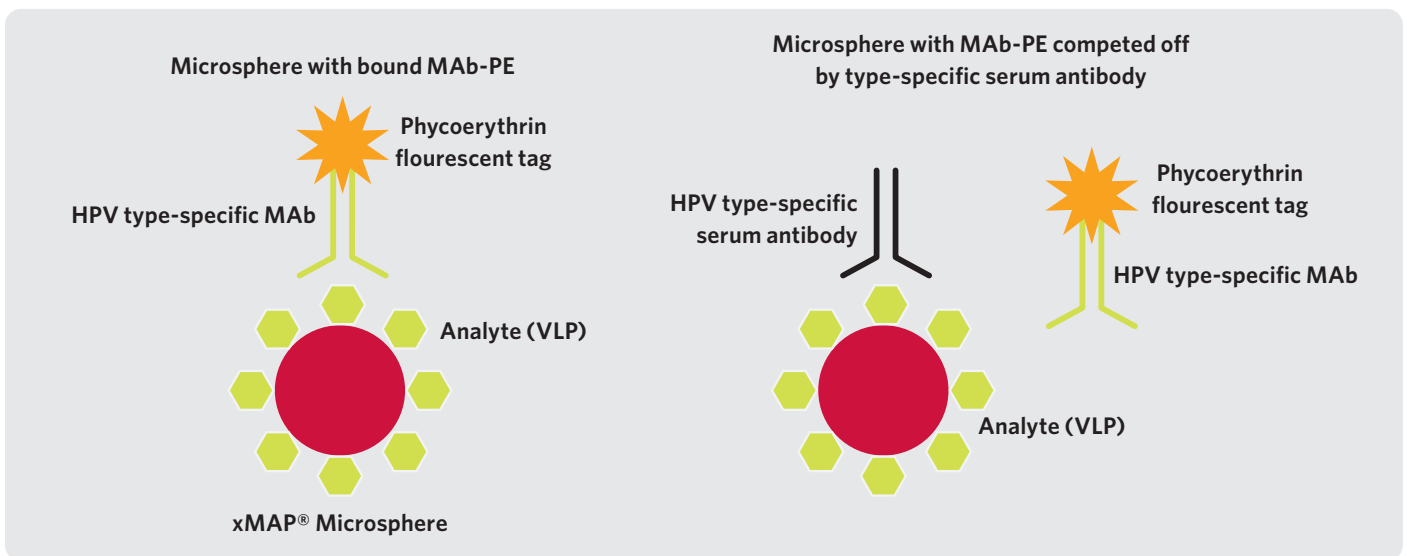


Development of the Gardasil® 9vHPV vaccine and the associated multiplex assay

The immense success of the Gardasil® 4VHPV vaccine fueled further vaccine development and clinical trials. The resulting 9vHPV vaccine protects against the original types of HPV targeted in the 4vHPV vaccine (6, 11, 16, 18), along with 5 additional oncogenic types of HPV (31, 33, 45, 52, and 58). As with the 4vHPV vaccine, xMAP Technology remained essential throughout the development of the vaccine and the testing of its safety and immunogenicity. Roberts et al. expanded the original 4vHPV cLIA[9-10] to a 9-plex format for use in clinical studies.[12] The resulting **9vHPV competitive xMAP immunoassay (9vHPV cLIA)** functions similarly to the 4vHPV cLIA assay, but with an additional 5 HPV VLPs coupled to microspheres.[12] The previously described **total IgG LIA** had already been developed to a 9-plex format and was

applied to studies and trials examining the 9vHPV Gardasil® vaccine.[11]

A total of 14,215 women aged 16-26 received either the 4vHPV or 9vHPV vaccine in a randomized, double-blind, international Phase IIb-3 study. The xMAP assays were central to the trial and were utilized to understand antibody responses as a primary outcome measure in the trial. The responses to HPV types 6, 11, 16, and 18 in people that received the 9vHPV vaccine were non-inferior to responses in the 4vHPV vaccine. The 9vHPV vaccine was also found to protect against the 5 additional types of HPV (HPV 31, 33, 45, 52, and 58).[40] The 9vHPV vaccine consequently received approval in 2014.[20]

A non-inferiority immunogenicity study helped bridge these findings to adolescent boys and girls aged 9-15 years. Three cohorts received the vaccine, including adolescent girls aged 9-15

years, adolescent boys aged 9-15 years, and young women aged 16-26 years. As in the initial study, the xMAP assays supported the immunobridging study as a primary outcome measure and helped support the vaccine's effectiveness. The 3,066 total subjects had >99% seroconversion 4 weeks after the third dose. Antibody responses in the adolescent boys and girls were non-inferior to responses in the 16-26 age group.[41] The initial findings were further bridged to older women 27-45 years of age. The xMAP assays were once again used as a primary outcome measure in the trial to demonstrate non-inferior antibody responses in women 27-45 years (n= 642) compared with women 16-26 years of age (n= 570).[42]

## xMAP Technology supports vaccine coadministration studies

Most worldwide vaccination schedules depend on concomitant vaccine administration. Administration of several vaccines at the same time increases vaccination uptake, improves adherence, and makes vaccination programs more economical.[43] xMAP Technology has supported studies exploring concomitant HPV vaccination with common childhood vaccines.

An early study in adolescent women examined concomitant administration of the 4vHPV vaccine with meningococcal conjugate vaccine (MCV4) and the tetanus, diphtheria, pertussis (Tdap) vaccine combination. The study randomized adolescent women between the ages of 10 and 17 years to receive either concomitant or non-concomitant vaccine administration with the 4vHPV vaccine. Administration with the 4vHPV vaccine was well-tolerated without any adverse impact on immune responses.[25, 44] The study concluded that concomitant administration was a valid strategy to minimize office visits and improve vaccine adherence.[25, 43-44] Similar results were also found examining 4vHPV and concomitant administration of hepatitis B vaccination[45] and Tdap with an inactive polio vaccine (Tdap-IPV).[25]

Likewise, an international study utilized xMAP Technology to measure seroconversion rates in boys and girls between the ages of 11 and 15 years during concomitant administration of the 9vHPV vaccine with MCV4/Tdap (n= 621) compared with a non-concomitant group receiving MCV4/Tdap alone (n= 620). Concomitant administration was well-tolerated and seroconversion rates were non-inferior compared with the non-concomitant group.[43] Both studies similarly concluded that concomitant administration was a valid strategy to minimize office visits and improve vaccine adherence.[25, 43-44]

## Alternative vaccination schedules

Vaccines that require multiple stringent doses are often more costly and associated with additional adherence problems.[46] Consequently, xMAP Technology has been used to explore the safety and efficacy of alternative vaccination schedules with the 4vHPV and 9vHPV vaccines. A recent literature review identified 23 studies examining the immunogenicity of alternative dosing schedules for HPV vaccination, many of which actively utilized xMAP Technology.[47]

For example, a randomized study utilized the total IgG LIA as a primary outcome measure to assess antibody response in girls that received a booster dose of either 4vHPV or 2vHPV after being previously vaccinated with two doses of the 4vHPV vaccine. The study concluded that both vaccines increased antibody titers and had acceptable safety profiles when given as booster vaccines.[46]

Studies continued to examine alternative dose regimens with the 9vHPV vaccine as well, eventually leading to regulatory approval of two-dose regimens 6-12 months apart for young adolescents that are still widely utilized today.[48-49] An ongoing study is further investigating this regimen to see if there were any negative repercussions to having extended intervals of 1-5 years between doses, which could enable more flexible dosing options for areas that have constrained resources and logistical hurdles.[49]

# Understanding long-term safety, immunogenicity, and the epidemiological impact of HPV vaccination

xMAP assays continue to be vital in post-licensure studies[50-54] and have helped demonstrate consistency between the immune responses of people receiving these vaccines and different vaccine lots.[55] Post-licensure initiatives of the 4vHPV vaccine have occurred in multiple countries worldwide, and many utilized the original 4vHPV cLIA and the total IgG LIA in efforts to demonstrate durable antibody responses and long-term safety.[50-52] A 10-year follow-up with the 3-dose regimen of the vaccine in pre-adolescents found durable responses and no new reported adverse events.[51] Meanwhile, a 14-year long-term safety follow-up of the 4vHPV vaccine in women from 4 Nordic countries also found no new reported medical conditions, and vaccinated individuals were found to have maintained high seropositivity rates as measured by xMAP assays.[52]

xMAP Technology was similarly used in post-licensure studies of the 9vHPV vaccine to understand the long-term antibody response and safety.[53-54] One study comparing a group of women given the 4vHPV vaccine to another group given the 9vHPV vaccine found a similar profile between the 2 vaccines 6 years after the first dose.[53] Meanwhile an 8-year follow-up in healthy girls and boys that received the 9vHPV vaccine also utilized xMAP Technology and found durable antibody responses and a well-tolerated, long-term safety profile.[54]

Finally, multiplex technology has been involved in epidemiological studies demonstrating the positive impact of vaccines on the incidence of HPV-associated diseases. A recent study examined vaccinated and unvaccinated subjects aged 0 to 75 years that were undergoing tonsillectomy for nonmalignant indications. xMAP assays were used to measure the prevalence of HPV to provide support that the UK's women-only vaccination program was associated with a reduction in oropharyngeal HPV-16 infections and possible herd immunity.[56] A similar study examining HPV vaccination in Swedish youth utilized the previously described xMAP assays to demonstrate a decreased prevalence of the HPV types the Gardasil® vaccines protects from over the course of 2008-2018. However, the study noted that other types of HPV remain a concern.[57]

## Summary

The 3 existing HPV vaccines are capable of preventing the vast majority of cervical cancers.[4] The 4vHPV and 9vHPV vaccines provide additional protection against types of HPV responsible for other diseases, including genital warts along with vulvar, vaginal, and anal cancers.[5-8] Traditionally, the development and testing of such vaccines would be time-consuming and costly, requiring running multiple tests to measure type-specific antibodies to different HPV genotypes.[9-12] However, xMAP multiplexing technology, especially the cLIA and total IgG LIA assays, has facilitated the development and clinical testing of these vaccines in a high-throughput and robust manner. These assays have facilitated the simultaneous measurement of type-specific antibodies to several HPV genotypes, and have been collectively used to measure titers in thousands of individuals.[9-12] These assays have also been used for drug registration, market release, and post-marketing surveillance. Although the vaccines have long since been approved, the multiplex assays are continuing to power studies examining long-term vaccine safety and efficacy, along with the worldwide impact of vaccination against HPV-related diseases.[56-57]

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