HPV INFECTION AND PERSISTENCE

STUDIES ON HPV INFECTION AND PERSISTENCE IN A UNIVERSITY UNDERGRADUATE POPULATION

By

ELISA BIBBY, B.Sc.

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AUTHOR: Elisa Bibby, B.Sc. (McMaster University)

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ABSTRACT

Cervical cancer is preceded by a spectrum of abnormalities in the cervical epithelium. Research supports an etiological role for certain types of human papillomaviruses (HPVs) in cervical pathology. More than 70 HPV genotypes have been characterized based on complete genome sequences and of these, about half infect the genital tract. Genital HPVs are further classified as either "high risk" or "low risk" types based on their association with cervical cancer. The demonstration that there is a relationship between HPV infection and cervical cancer has been dependent on a number of viral nucleic acid-based detection systems such as Southern blot and polymerase chain reaction. However, the lack of methods which discriminate between a specific HPV type and a large number of related HPV genotypes has made studies of disease association difficult.

In the first part of this study, a recently developed Amplicor HPV Genotyping Kit was evaluated with respect to its ability to define HPV infection status. The L1 region was directly sequenced from the PCR product in 16 clinical samples to determine which genotype(s) was/were present. Sequencing data from one sample suggested a mixed infection and therefore the PCR product was cloned and sequenced to see if more than one genotype was present. Fifteen out of the sixteen samples sequenced were HPV genotypes which are not represented on the Amplicor Genotyping Kit test strips. The

samples are rare HPV types (HPV 61, 62, CP6108 and CP8304).

The second part of this study examined the issue of persistence and in particular, I have considered the issue of an appropriate definition of persistence. A number of patients who had evidence of HPV 16 or HPV 18, 6 and 66 were investigated using samples obtained on at least two occasions. Molecular variants of either the LCR gene for HPV 16 or the L1 gene for the other HPV types were studied. No differences in LCR or L1 gene sequence in sequential same patient samples were observed . Two HPV 16 LCR alleles were seen for six patients of which 8/10 showed one nucleotide change at base 7518 and 2/10 were identical to the prototype. Based on a compilation of published studies on HPV 16 variants and subtypes for the long control region, the number of HPV 16 LCR alleles, worldwide, was determined. One HPV 66 and two HPV 18 and HPV 6 L1 alleles were observed. When examining persistence, one must consider the frequency of specific variants (alleles) in the study population. A common variant detected over time could either be a persistent infection or a reinfection with the same viral variant.

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LIST OF ABBREVIATIONS

ATP	adenosine triphosphate
bp	base pairs
°C	degrees Celsius
CIN	cervical intraepithelial neoplasia
CIS	carcinoma *in situ*
dATP	deoxyadenosine triphosphate
dCTP	deoxycytosine triphosphate
dGTP	deoxyguanosine triphosphate
dTTP	deoxythymidine triphosphate
dNTPs	deoxynucleotide triphosphates
DNA	deoxyribonucleic acid
E	early
EV	Epidermodysplasia Verruciformis
G_o	G- "zero" phase (cell cycle phase)
G_1	Gap 1 phase (cell cycle phase)
G_2	Gap 2 phase (cell cycle phase)
HPV	human papillomavirus
IPTG	isopropyl-1-thio-β-D-galactoside

kb	kilobase
L	late
LB	Luria-Bertani
LCR	long control region
M	mitosis (cell cycle phase)
ORF	open reading frame
PCR	polymerase chain reaction
S	synthesis phase (cell cycle phase)
SB	southern blot
Taq	Thermus Aquaticus
TAE	Tris-Acetate EDTA
TBE	Tris-Borate EDTA
UV	ultraviolet
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactoside
g	gram
L	litre
m	milli
M	moles per litre
n	nano
p	pico
μ	micro

INTRODUCTION

I. Cervical Cancer

About 500,000 women worldwide die each year from cervical cancer. The same number of cases are diagnosed every year, mostly in developing countries, however the actual number of cases is thought to be much greater (Braly, 1996; Shah *et al.*, 1996). Cervical cancer is one of the most prevalent cancer in women worldwide, second only to breast cancer (Favre *et al.*, 1997). According to the American Cancer Society, Atlanta, Georgia, 1999, 13,700 new cervical cancer cases were diagnosed and 4900 deaths occurred in 1998 (American Cancer Society: American Cancer Statistics, United States of America, 1999). In Canada, it is estimated that in 1999, 420 deaths will occur and 1500 new cervical cancer cases will be diagnosed (National Cancer Institute of Canada: Canadian Cancer Statistics, Toronto, Canada, 1999).

Geographical differences in cervical cancer prevalence are apparent. The highest incidence occurs in developing countries. This is primarily due to the lack of effective screening methods in these countries (Birley, 1995). However, despite effective screening methods and medical intervention in developed countries, cervical carcinoma is increasing among young women. This increase is attributed to several factors including lack of sexual education, inadequate use of contraceptives, multiple sex partners and early

onset of sexual activity.

I b. The Cervix and Cervical Malignancies

The human cervix is a cylinder of fibromuscular tissue averaging about 3.5 cm in length and 2.5 cm in diameter. The upper and middle thirds of the cervical canal are lined by columnar epithelium continuous with the endometrium, the mucous membrane lining the inner surface of the uterus, proximally. The peripheral portion of the ectocervix is covered by squamous epithelium (Reid, 1993). The region where the squamous and columnar epithelium meet is known as the squamo-columnar junction. In young pubertal women, the squamo-columnar junction is found at the cervical os on the external portion of the cervix. Throughout reproductive life, there is gradual ascent of this junction up the endocervical canal (Garland *et al.*, 1992). The normal columnar epithelium is gradually and permanently replaced by squamous epithelium. This area of squamous metaplasia is called the transformation zone (Reid, 1993). It is thought that a predisposition to oncogenesis occurs at this unstable transformation zone as opposed to the stable epithelium found elsewhere.

Nearly all cervical cancers originate in the transformation zone. The high incidence of cervical cancer as compared to the low incidence of cancer at other sites in the female lower genital tract (vagina, vulva, perineum) is ascribed to the high susceptibility of the transformation zone to human papillomaviruses and other cofactors (Shah *et al.*, 1996).

Invasive cervical cancer is preceded by a progressive spectrum of abnormalities of the cervical epithelium. These precursor lesions of invasive squamous carcinoma, which is the predominant cervical malignancy, are commonly termed cervical intraepithelial neoplasia (CIN). There are three grades of CIN: CIN I (mild dysplasia), CIN II (moderate dysplasia) and CIN III (severe dysplasia/ carcinoma in situ (CIS)). The three grades are determined largely by the proportion of the epithelium occupied by undifferentiated basal cells; less than one third (CIN I), one third to two thirds involvement (CIN II) and two thirds (CIN III) to full thickness (CIS) respectively (Garland *et al.*, 1992). The risk of malignancy increases with the progression of CIN I to a higher grade dysplasia (Koutsky *et al.*, 1992). The time interval between early cervical abnormalities and invasive carcinoma may span several decades. During this long interval, cytological abnormalities are detected by Papanicolaou (Pap test) and can be treated (Shah *et al.*, 1996).

I c. Cervical Cancer, Sexually Transmitted Diseases and Human Papillomavirus

For more than a century, sexual activity has been recognized as one of the most important risk factors involved in the development of cervical carcinoma. Therefore, a search for a causative factor that can be sexually transmitted has been thoroughly investigated. Several infectious agents have been considered for this role. Herpes Simplex Virus Type 2 (HSV-2), at one time, was considered the oncogenic agent for cervical cancer. However, the absence of HSV-2 DNA from most tumours as well as

numerous epidemiology studies show that it does not play a primary role in the development of cervical cancer (zur Hausen, 1976; DiPaolo *et al.*, 1990).

In 1976, zur Hausen suggested that the sexually transmitted agents that infect the genital tract and cause genital warts may play a role in genital cancers (zur Hausen, 1976). Since then, research and data has accumulated rapidly to support the etiological role for the certain types of human papillomaviruses (HPVs) in cervical cancer and its precursors. In 1996, a consensus panel convened by the National Institutes of Health concluded that cervical cancer is 'causally related' to HPV infection (McNeil, 1996).

II. Human Papillomaviruses

Human Papillomaviruses are non-enveloped double stranded DNA viruses that exhibit a specific tropism for epithelial tissues. These particles consist of an icosahedral capsid which contains a circular genome that is approximately 8000 base pairs in length (Shah *et al.*, 1996). HPVs belong to the Papillomavirus genus of the family Papovaviridae. Papillomaviruses are widespread among vertebrates (Bonnez, 1997).

The first papillomavirus was isolated by Richard Shope in 1933 (Shope *et al.*, 1933) and is known as the cottontail rabbit papillomavirus. Shope described this virus of wild type origin as readily transmissible to domestic rabbits. In animals, the infection is associated predominately with cutaneous epithelial lesions (Howley, 1996).

Papillomaviruses demonstrate species specificity with respect to infection and replication and thus, do not cross-infect other species. HPVs only infect epithelial tissues

of humans (Shah *et al.*, 1996). In humans, complete genomes of more than 70 types have been characterized (de Villiers, 1997; http://hpv-web.lanl.gov/).

Three broad HPV classes exist based upon their site of isolation; genital-mucosal, non-genital and Epidermodysplasia Verruciformis (EV) which is a rare skin condition in which patients develop chronic cutaneous HPV lesions. The genital HPVs are further classified as "high risk" and "low risk" based on their association with cervical cancer. Low risk types, such as HPV 6 and 11, are almost never found in cervical malignancies but have been isolated from external genital warts. HPV 16 and 18 are most frequently identified in cervical cancers and are therefore classified as high risk but there are several other types including 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 (Lowy *et al.*, 1994; Howley *et al.*, 1996; Bonnez, 1997) which are isolated less frequently.

II b. Viral Genome Organization

The viral genetic information is encoded on only one of the two DNA strands which contains at least eight open reading frames (ORFs) divisible into three regions: a long control region (LCR) corresponding to 7-11% of the genome length, an early (E) region (50% of genome) and a late (L) region (Favre *et al.*, 1997). The early and late regions assignments are based on time of expression over the course of a productive infection. All papillomaviruses have a similar genetic organization (see Figure 1).

The early region codes for regulatory functions that are responsible for viral DNA replication and cellular transformation and these genes are expressed in non-productively

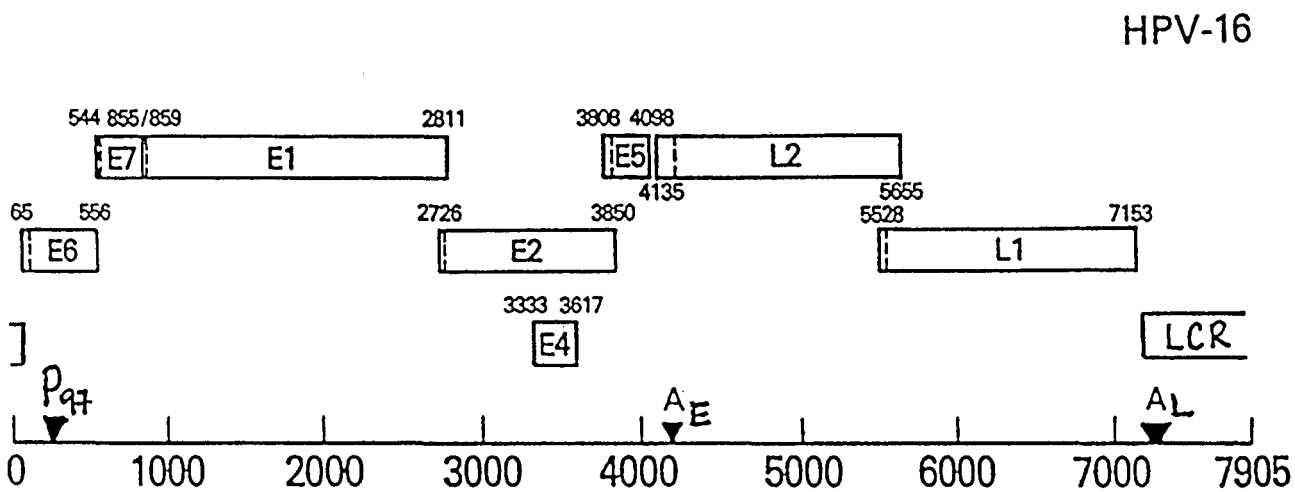

FIGURE 1: HPV 16 GENOME ORGANIZATION

A linearized version of genome with open reading frames (ORFs) which are indicated by numbered boxes. Nucleotides are numbered based on the genomic organization of the HPV 16 (Seedorf *et al.*, 1985). This map includes the correction for the frame shift in the E1 ORF and for the E5 ORF.

E = early; L = late; A = polyadenylation sites; LCR = long control region; P = promoter (Howley, 1996).

infected cells. The late region encodes structural proteins and is expressed in productively infected cells (Howley *et al.*, 1996; Shah *et al.*, 1996; Favre *et al.*, 1997).

The early region consists of six different ORFs, E1-E7 which encode non-structural proteins involved in cell transformation (E5, E6, E7) or in the replication and transcription of the viral genome (E1, E2). The L region encodes L1 and L2, the major and minor capsid proteins respectively (Favre *et al.*, 1997). E4 is expressed as a late gene and is involved in the maturation and release of the virus (Villa, 1997). The functions of the viral proteins are summarized in Table 1.

The LCR, also known as the non-coding region and upstream regulatory region, contains the viral origin of replication and transcriptional responsive elements that regulate HPV gene expression. It is positioned between the end of the L1 gene and the start of the E6 gene and contains no open reading frames. The LCR region does not show extensive nucleotide similarities between closely related genotypes, however there are a number of short motifs that are highly conserved in related HPVs (Howley *et al.*, 1996; Villa, 1997).

TABLE 1 : FUNCTIONS ASSIGNED TO THE PAPILLOMAVIRUS OPEN READING FRAMES AND NUCLEOTIDE NUMBERS

ORF	NUCLEOTIDE NUMBERS eg. HPV 16 L= 7905bp	FUNCTION
L1	5528-7153	L1 protein, major capsid protein
L2	4135-5655	L2 protein, minor capsid protein
E1	859-2811	initiation of viral DNA replication
E2	2726-3850	transcriptional regulatory protein with an auxiliary role in viral DNA replication
E3		no known function
E4	3333-3617	late protein, disrupts cytokeratins
E5	3808-4098	membrane transforming protein; interacts with growth factor receptors
E6	65-556	transforming protein of HPVs; targets degradation of p53
E7	544-855	transforming protein of HPVs; binds to the retinoblastoma protein

ORF= open reading frame; HPV= human papillomavirus; E= early; L= late (Howley, 1996 and Shah *et al.*, 1996).

II c. Replication Cycle

Only basal, replicating keratinocytes are infected by HPVs and viral infection must occur initially after access through an epithelial layer where the integrity has been compromised by wounding or abrasion (Alani *et al.*, 1998). The normal replication cycle of HPVs is a highly regulated process which is dependent upon both the virus encoded proteins and the degree of differentiation of the host epithelial cell (Wright *et al.*, 1990). Replication occurs exclusively in the nucleus of the infected cell (Alani *et al.*, 1998). Although a viral receptor on host cells has not been definitively identified, an $\alpha 6\beta 4$ integrin of basal keratinocytes has been suggested as a candidate receptor (Evander *et al.*, 1997; Alani *et al.*, 1998). In the first stage of replication, viral genomes are maintained in deeper epithelial layers as multicopy nuclear episomes that replicate in S phase with the host chromosome. The early region is transcribed at low levels. E1 and E2 are required for HPV DNA synthesis and E5, E6 and E7 induce proliferation of the basal and parabasal cells. The next stage takes place during the terminal differentiation of keratinocytes as they migrate towards the surface. Vegetative viral DNA replication, E4 expression, L1 and L2 synthesis and assembly occur at the same time (Favre *et al.*, 1997). Refer to Figure 2.

The HPV genome can exist either extrachromosomally as a plasmid (episome) or integrated into the host genome (Alani *et al.*, 1998). Integration into the host genome is not an obligatory step in the life cycle, although it has been described frequently in HPV 16 and HPV 18 infected cells (Cullen *et al.*, 1991; Stoler *et al.*, 1992; Kristiansen *et al.*,

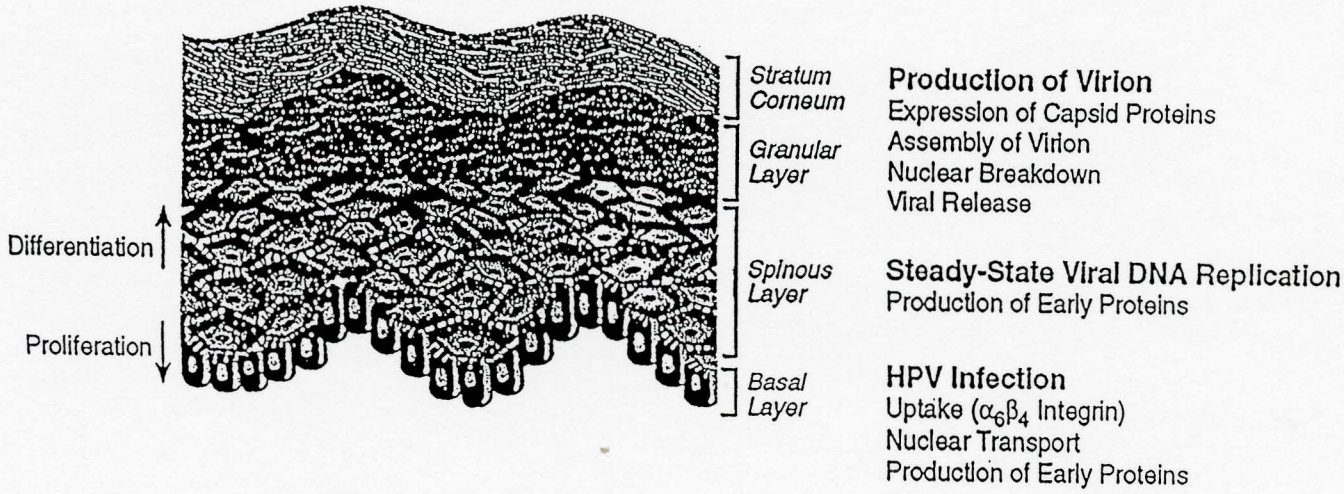

FIGURE 2: REPLICATION CYCLE

The human papillomavirus viral life cycle within stratified squamous epithelium (Alani *et al.*, 1998)

1994). In a 1991 study, Cullen *et al.* demonstrated that 56 out of 69 (81%) of cervical carcinomas showed integrated DNA. In particular, 72% of carcinomas containing HPV 16 DNA had integrated DNA whereas all of the HPV 18 containing carcinomas had integrated DNA. Kristiansen *et al.* (1994) showed that integration of HPV 16 was found in all cervical carcinomas. The site of integration appears to be random given that different chromosomal insertion sites have been characterized in cancers and cell lines (Park *et al.*, 1995). In certain cell lines, integration has occurred near cellular oncogenes such as n-*myc* or c-*myc* (Couturier *et al.*, 1991). This event may activate these cellular oncogenes leading to malignant progression.

Specificity does occur in the location of the breakpoint within the HPV genome. Integration generally disrupts the E1-E2 region which knocks out negative regulatory control of the E6/E7 genes. This is thought to confer a growth advantage in tumour cells and integration of the viral DNA into the cell genome contributes to the increase of E6/E7 transcription during tumour progression (Cullen *et al.*, 1991; Romanczuk *et al.*, 1992). In some cases, the late region can be lost during the integration process (Resnick *et al.*, 1990; Tate *et al.*, 1996).

In HPV-infected benign lesions, the viral DNA is usually found in an episomal state whereas in carcinomas, the viral DNA is usually integrated into the host genome with episomal DNAs in high copy numbers. It has therefore been suggested, but not confirmed, that this integration event is a prerequisite for malignant conversion in genital tumours (Cullen *et al.*, 1991; Kristiansen *et al.*, 1994). The long latency period between

the initial HPV infection and cancer development suggests that HPV infection alone is insufficient for carcinogenesis. Also, only a small proportion of women infected with cancer causing HPV types will develop invasive cervical cancer. Additional cofactors and cellular events are therefore probably required for cervical carcinogenesis (Park *et al.*, 1995).

HPVs have been difficult to study in the laboratory because of the small number of tissue culture systems that allow these viruses to replicate. This is due to the dependency of the viruses on epithelial differentiation (Bedell *et al.*, 1991). In recent years, an *in vitro* system has been developed, the raft culture, which allows primary human foreskin keratinocytes to achieve replication and differentiation morphologically similar to normal foreskin. Keratinocytes are seeded on a porous collagen gel containing fibroblasts and at confluence, the assembly is raised to the air-liquid interface (Dollard *et al.*, 1992; Meyers *et al.*, 1992). Dollard *et al.* used raft culture to obtain HPV 11 growth while Meyers *et al.* successfully propagated HPV 31.

II d. Cellular Transformation and E6/E7

Experimental studies support the observation that high risk HPVs play a role in the development of cervical cancer. Specifically, these studies point to the central transforming ability of the E6 and E7 viral oncogenes. High levels of E6 and E7 transcripts are found in HPV positive cancers (Stoler *et al.*, 1992). Münger *et al.* (1989) showed that the E6 and E7 genes of HPV 16, together, are necessary and sufficient for the

transformation of human keratinocytes.

E6 and E7 are multi-functional proteins (Alani *et al.*, 1998). The E6 protein has been shown to form complexes with the tumour suppressor protein, p53 (Werness *et al.*, 1990) which plays a central role in cell cycle regulation and growth suppression. After forming a complex with p53, the HPV E6 protein promotes degradation (Scheffner *et al.*, 1990) through the ubiquitin-dependent proteolytic pathway (Scheffner *et al.*, 1993).

The E7 protein binds preferentially to the retinoblastoma gene product, Rb, and its related proteins, p107 and p130 (Dyson *et al.*, 1989; Park *et al.*, 1995). The Rb protein exists in two forms depending upon the stage of the cell cycle. A phosphorylated inactive form is found in the S, G_2 and M stages and a hypophosphorylated, active form predominates in the G_0 and G_1 stages. In normal cells, the hypophosphorylated forms of the Rb protein, p107 and p130, form complexes with transcriptional factors of the E2F family. These hypophosphorylated complexes inhibit cells from entering the S phase of the cell cycle and therefore negatively regulate cell growth by repressing transcription. As cells progress through the cycle, the E2F-Rb complexes dissociate and the free E2F stimulates transcription and allows DNA replication (Park *et al.*, 1995). The E7 protein binds to Rb and dissociates the E2F-Rb complex and thus releases and activates the transcription factor E2F. This stimulates the transcription of genes necessary for DNA replication (Chellappan *et al.*, 1992; Park *et al.*, 1995).

These activities for E6 and E7 are much greater for high risk HPV types compared to low risk types (Barbosa *et al.*, 1990; Crook *et al.*, 1991; Münger *et al.*, 1991).

Specifically, the E7 protein from the "high" risk HPV types demonstrate a higher affinity for Rb than do E7 proteins from the "low" risk HPV types. Furthermore, the E6 protein of both types bind with p53, however only the "high" risk types target p53 for degradation.

III. Classification of Papillomaviruses

Unlike other virus families, HPVs are not subdivided into serotypes based on structural antigenic features recognized by antibodies. Capsid proteins of papillomaviruses are antigenically similar and serology is problematic. In addition, most HPVs cannot be grown *in vitro* in sufficient quantities for biochemical and antigenic characterization. Therefore, HPVs are subdivided into genotypes and subtypes based on the extent of DNA homology between isolates (Park *et al.*, 1995; Bonnez, 1997). Sequencing of HPV isolates has revealed regions of low homology and regions with high homology within the L1, E1, E6 and E7 genes. In the past, an isolate was defined as a new type when the DNA sequence of the L1, E6 and E7 ORF shared a homology of less than 90% when compared to the closest related known HPV type. However, the present definition of a genotype is defined as an HPV that shares homology of <90% to the closest related papillomavirus type in the L1 ORF only (de Villiers, 1997). A subtype shares 90-98% of sequence similarity while a variant has >98% DNA sequence identity (Favre *et al.*, 1997). More than 130 HPV types have been identified based on PCR amplification products from the L1 ORF (de Villiers, 1997). HPV genotypes, variants

and subtypes have been published and are updated frequently at http://hpv-web.lanl.gov/.

III b. Genomic Variability of Papillomaviruses

Over the last decade, with the availability of large amounts of papillomavirus molecular sequence information, we have been able to analyze this information in terms of the process of molecular evolution. The phylogenetic trees are derived from extensive nucleotide comparisons and reflect the relationship between papillomaviruses and their hosts (Chan *et al.*, 1992, 1995; Stewart *et al.*, 1996). Chan *et al.* (1995) grouped all papillomaviruses into five major branches, called supergroups, of which two branches represent animal PVs and the other three branches are human PVs. The largest supergroup contains all genital HPVs and some non-genital HPVs, mostly grouped on the basis of partial L1 and E6 nucleotide sequence information.

Sequence variability has been well documented in certain HPVs. The most detailed studies of HPV variability have been done with HPV 16 (Ho *et al.*, 1991, 1993a, 1993b; Icenogle *et al.*, 1991; Chan *et al.*, 1992; Eschle *et al.*, 1992; Yamada *et al.*, 1995; Xi *et al.*, 1995, 1997; Wheeler *et al.*, 1997), which is the most common genotype. HPV 16 has evolved along five phylogenetic branches; European, Asian, Asian-American and two African (Chan *et al.*, 1992). Ho *et al.* (1993a) suggest that HPVs have coevolved with their human hosts over a period of several million years.

Nucleotide sequence variation has been an important consideration in epidemiological studies of viral transmission and persistence and has complicated viral

type identification protocols.

IV. HPV Detection Methods

Demonstration of a relationship between HPV infection and cervical cancer has been dependent on a number of detection methods. Although cytologic and histologic methods are used in clinical diagnostics, association of particular HPV types with risk of disease has not been confirmed using these techniques alone. Therefore, detection of HPV and type specific identification of HPV in clinical specimens has relied entirely on nucleic acid detection assays including *in situ* hybridization, dot blot or slot blot, Southern blot (SB) and the polymerase chain reaction (PCR). These first three methods are laborious, time consuming and often lacking in specificity and sensitivity when compared to PCR (Saiki *et al.*, 1988; Dallas *et al.*, 1989; Bauer *et al.*, 1991; Tham *et al.*, 1991; Yoshikawa *et al.*, 1991).

Dallas *et al.* (1989) examined HPV types in cervical specimens and biopsies using PCR and were able to detect as few as 10 copies of virus (0.08 fg DNA). Tham *et al.* (1991) compared sensitivities of southern blot and PCR. Southern blot hybridization sensitivity was 1 pg or approximately 10^5 molecules of HPV DNA whereas PCR was effective in identifying as few as 10-100 HPV DNA molecules. Several studies have indicated that PCR is more sensitive than SB, especially in cases with low amounts of HPV DNA (Chang *et al.*, 1995; Hoffmann *et al.*, 1998). Conventional Southern blot hybridization had been considered the gold standard for determinations of virus infection

but this has been almost completely replaced by amplification of the viral DNA by PCR (de Villiers, 1997).

PCR is a rapid and sensitive method with enzymatic amplification of a specific DNA sequence up to a millionfold. In the past, cross-contamination of samples and standards may have been problematic and gave rise to false positives and therefore an overestimate of viral prevalence (Dallas *et al.*, 1989). Adoption of stringent lab procedures and inclusion of several controls has eliminated cross-contamination problems. A 1998 study, which examined the accuracy of PCR analysis for HPV detection, demonstrated that the false negative rate in HPV testing by PCR for precancerous lesions of the cervix was 0% (Zazove *et al.*, 1998). These results illustrate that PCR is an appropriate test for the identification of women with HPV-related lesions of the cervix.

Two basic PCR detection strategies use either type-specific or consensus primers. However, given the large number of HPV genotypes, variants and subtypes, and potential new types still to be identified, the type-specific primer strategy becomes less cost efficient, less sensitive and more laborious. On the other hand, consensus primers, based on conserved regions of the HPV genome, allow the detection of both known and novel HPV types (de Villiers, 1997). Theoretically, consensus primers should identify all HPV-infected samples. In a 1993 study (Coste-Burel *et al.*, 1993), a comparison was done between consensus and type-specific primers for HPV identification in lesions of various grades. They noted that 10% of HPV 16 positive samples were not detected by Gregoire

consensus PCR primers (Gregoire *et al.*, 1989) and concluded that both PCR techniques should be used. A study in our laboratory was initiated because, we too, found Gregoire primers (Gregoire *et al.*, 1989) unable to identify samples that were HPV positive by type-specific primers. We evaluated five sets of commonly used consensus primers [Manos (Manos *et al.*, 1989), Gregoire (Gregoire *et al.*, 1989), Snijders (Snijders *et al.*, 1990), van den Brule (van den Brule *et al.*,1990), Yoshikawa (Yoshikawa *et al.*, 1991)] for HPV detection. Although the Yoshikawa primer pair (Yoshikawa *et al.*, 1991) which amplifies the L1 region clearly performed better than the others, the data indicated that PCR typing for HPVs requires more than one consensus primer pair to identify all HPV-infected samples. In particular, a combination of Manos (Manos *et al.*, 1989) and Yoshikawa (Yoshikawa *et al.*, 1991) primers was best able to detect all HPV positive samples (Harnish *et al.*, 1999).

Recently, a reverse blot method was developed for HPV genotyping using a L1 consensus PCR product which is hybridized to an array of HPV DNA probes and β-globin control probes. This convenient method allows typing of twenty-seven genotypes in one reaction (Gravitt *et al.*, 1998). Coutlée *et al.* (1999) compared and evaluated this line blot assay to a standard PCR test, in which amplified products were detected in a dot blot assay using radiolabeled type-specific probes. Initially, the agreement between the two assays was 83.9% (214 out of 255 samples). In four of the discordant samples, the line blot assay detected genotypes unable to be detected by dot blot and in the other thirty-seven samples, the dot blot assay detected types undetected by the line blot assay.

Discordant samples were reexamined without coamplification with β-globin primers. Thirty-three out of thirty-seven discordant samples were resolved. With modifications, the line blot assay compared favorably to the dot blot assay.

Although typing is currently not used in patient management, Lombard *et al.* (1998) showed that HPV genotype is a major determinant in the course of cervical cancer. In particular, HPV 16/18 is strongly associated with CIN II/III. In addition, the number of HPV genomes in cells has been shown to correlate with the severity of cervical disease. Two studies have shown that high levels of HPV 16 almost always predicted CIN II/III lesions whereas low level infections were not helpful in this regard. A PCR based protocol was used to identify the levels of DNA (Bavin *et al.*, 1993; Cuzick *et al.*, 1994).

V. Viral Persistence and Disease Progression

HPV can remain latent without stimulating cellular differentiation and wart production. This may be observed when primary infection does not immediately lead to a wart or when wart regression is followed by wart formation which may be induced at later times by either immunosuppression or other stimuli. Little is known about the mechanism(s) involved in establishing or maintaining a latent infection (Steinberg *et al.*, 1983; Ferenczy *et al.*, 1985; Ahmed *et al.*, 1996).

Preliminary data suggests that the majority of HPV infections are found to be transient and only a small proportion of women tend to harbour the same HPV type in

cancer, she is more likely to develop cervical intraepithelial neoplasia than when transiently infected with HPV (Koutsky *et al.*, 1992; Hildesheim *et al.*, 1994; Remmink *et al.*, 1995; Londesborough *et al.*, 1996; Moscicki *et al.*, 1998; ter Harmsel *et al.*, 1999).

Recently, ter Harmsel *et al.* (1999) concluded that persistent infection with HPV 16 is associated with a higher risk of developing CIN. In addition, the CIN that develops is more severe and often high grade when compared to women who are transiently infected with HPV 16. These results are comparable to the observations of Koutsky *et al.* (1992) and Remmink *et al.* (1995) who examined the relationship between HPV 16 or HPV 18 infection and persistence. They concluded that persistence of high risk types leads to the progression of cervical lesions and that this is not observed in the presence of low risk HPVs or in the absence of HPV. Ho *et al.* (1995) observed that high risk type-specific persistent HPV infection, particularly with high viral load, correlates with chronic cervical dysplasia. In a 1996 study, Chua *et al.* examined archival Pap smears of patients who were diagnosed with HPV-containing cervical carcinoma. They demonstrated similar results; persistent HPV infection of high risk types 16 and 18, can be shown many years before diagnosis of clinical disease.

To address the question of persistence, many studies have focused on the analysis of molecular variants of HPV using more refined DNA sequence analysis. Variants have been examined for mutational patterns in the viral DNA. Londesborough *et al.* (1996) showed that there is an association between persistence and a variant of HPV 16 which has a base change at nucleotide 350 in the E6 gene region. In a more recent study, Xi *et*

al. (1997) showed that non-prototypic-like HPV 16 variants confer a greater risk in the development of CIN II/III than prototypic-like variants. This study was conducted among women attending university and women who attended a sexually transmitted disease clinic. These results combined with Zehbe *et al.* (1998), who showed that HPV E6 variants are more prevalent in invasive cervical carcinoma than the prototype, suggest that the underlying genomic differences may be important in the progression of HPV infection to cervical cancer.

Ho *et al.* (1991) showed, by sequencing part of the viral LCR (the enhancer), that HPV 16 variants have many mutations which occur multiple times and in several combinations. The LCR may differ dramatically between different HPV genotypes. Two groups have studied the diversity of HPV 16 by sequencing the LCR from many samples. Chan *et al.* (1992) reported thirty-eight variants from 118 HPV 16 isolates, most of which differed by one or several point mutations. Xi *et al.* (1993) found twelve HPV 16 variants in 48 anal specimens and seven variants in 10 cervical carcinoma samples.

Xi *et al.* (1995) also analyzed sequence variation in the LCR region. However, they sequenced a large number of clones from a smaller number of specimens; more than one HPV 16 variant in an individual was observed in several cases. This confirmed the observations of Ho *et al.* (1991) and Xi *et al.* (1993) but they also found a number of HPV 16 variants distributed among three cohort populations. One of these variants persisted over time while the other variants were only transiently detected. This may indicate an advantage of one variant over another that would be important in disease

progression. Several other studies also showed the presence of more than one HPV 16 variant in an individual (Ho *et al.*, 1993b; Chan *et al.*, 1992; Xi *et al.*, 1995; Wheeler *et al.*, 1997). Franco *et al.* (1994) and van Belkum *et al.* (1995) also sequenced this region. Both of these groups found persistently infected women who had no differences in HPV 16 LCR sequence, indicating that viral persistence may be a more common feature than previous thought. Of course, at this point, it is not clear if definitions of viral persistence based on sequence identity are appropriate or whether a case can be made for ignoring sequences and refocusing on infection with any HPV as the clinically relevant feature.

Objectives of the study:

This study had two objectives. The first was to evaluate the recently developed Amplicor line blot detection method. In order to do this, we studied HPV clinical samples from our extensive HPV database which had been typed by other methods. Specifically, most of the samples that were looked at were previously positive by ethidium bromide and/or dot blot but previously negative by Amplicor strip. In order to complete this first objective, a part of the L1 region was sequenced. This was done to determine which HPV genotype(s) is/are present in the clinical samples and to further ascertain whether these HPV samples should have been positive by Amplicor strip analysis. The second objective of this study was to examine the issue of persistence of HPV. This required the sequencing of the variable long control region for HPV 16 infected samples. For other HPV genotypes, the L1 region was sequenced. The results of

studies which address the first objective are described in Chapter 1 and the second objective is examined in Chapter 2.

MATERIALS AND METHODS

Data Collection and Clinical Specimen Preparation

More than 3500 clinical samples were collected from cervical swabs of women attending the University of Toronto Health Service Clinic for non-gynecological concerns. These women consented to participate in an HPV study and filled out an extensive risk-factor questionnaire. 1573 of our samples are from the first visit (baseline) and a subset of these patients returned for follow-up visits one and two years after their initial visits. The cervical cells were processed as previously described (Rohan *et al.*, 1991; Harnish *et al.*, 1999).

Samples were prepared and HPV typing was done by Elizabeth Scheid (McMaster University, Department of Pathology). HPV was detected using the polymerase chain reaction (PCR) using type-specific oligonucleotide primers (6/11, 16, 18, 33) as well as consensus primers, MY09 and MY11, which amplify a 450bp fragment in the L1 region (Manos *et al.*, 1989). All samples were initially amplified with beta-globin primers (amplimer size = 280bp) to ensure that there was DNA present which was capable of being amplified.

Following amplification, aliquots from PCR reactions were separated by gel electrophoresis on agarose gels (2% NuSieve, 1% agarose) using ethidium bromide and

examined under UV light. A number of the Manos amplified reactions (all baseline) were also assessed by dot blotting. Aliquots from PCR reactions were blotted onto membranes and hybridized to a probe cocktail containing α-[^{32}P]-dCTP labelled HPV 6, 11, 16, 18 and 33 DNA. Amplimers were detected by autoradiography after washing at 55°C and 65°C.

Recently, E. Scheid reassessed study samples using an Amplicor HPV Genotyping Kit by Roche Molecular Systems. Samples are amplified with biotinylated Manos primers and denatured PCR products are hybridized to an array of 27 HPV probes plus two beta-globin control probes which are immobilized in lines on strips. Detection of hybrids requires a colour reaction (Gravitt *et al.*, 1998; Coutlée *et al.*, 1999).

Oligonucleotide Primers for PCR

Manos HPV consensus primers and type-specific HPV 16 long control region primers were synthesized by The Central Facility of the Institute for Molecular Biology and Biotechnology, McMaster University according to the published sequences (Manos *et al.*, 1989; Ho *et al.*, 1991). All primers were 20 nucleotides in length and only the Manos primers were degenerate in their design. This permits alternate base paring to occur at certain positions. Manos oligonucleotide primer sequences are localized within the L1 ORF. However, their location varies with HPV genotype.

Manos	L1 ORF	Amplimer size: 450bp
Primer 1	5' GC A/C CAG GG A/T CAT AA C/T AAT GG 3'	
Primer 2	5' CGT CC A/C A A/G A/G GGA A/T AC TGA TC 3'	

HPV 16 (type-specific)	LCR	Amplimer size: 404bp
Primer 1	5' TCG GTT GCA TGC TTT TTG GC 3'	
Primer 2	5' CGG TTT GCA CAC ACC CAT GT 3'	

PCR Amplification

Several precautions were taken to reduce the possibility of contamination. Clinical samples were processed in 4H13. All materials (stuffed pipette tips, glassware, Eppendorf tubes) and reagents were autoclaved prior to use for PCR and kept in a separate PCR room. Pipettemen, racks, gloves, a microcentrifuge and any other solutions or equipment that were needed were kept in this same room. Disposable gloves were worn at all times and changed frequently during the preparation of samples or reaction mixes. Reaction mixes and reagents were prepared in a laminar flow hood. Amplification and analysis of PCR products occurred in a different location from where clinical sample processing took place. Pre and post amplification samples were also kept physically separated and all amplified samples were stored in separate freezers from the non-amplified clinical samples. HPV positive controls were stored separately from unamplified clinical samples.

The polymerase chain reaction was used as described elsewhere (Saiki *et al.*, 1988, Dallas *et al.*, 1989). Reactions were carried out in 100μL final volumes in a 0.5mL microcentrifuge tube. For Manos consensus primers, reactions contained 20μL of sample, 1X GeneAmp PCR Buffer II (10mM Tris-HCl pH 8.3, 50mM KCl), 2.0 mM $MgCl_2$, 0.1 mg/mL Bovine Serum Albumin, 0.2mM dNTPs (dATP + dTTP + dCTP + dGTP), 0.5μM of each primer and 2 units of Taq DNA polymerase (AmpliTaq™, Perkin-Elmer, Cetus). The volume was adjusted with distilled sterile water. The reactions were carried out for 35 cycles of amplification in a thermal cycler (Perkin-Elmer, Cetus). These cycles consist of 1 minute at 94°C for denaturation , 2 minutes at 55°C for annealing and 2 minutes at 72°C for elongation. In the last cycle, elongation at 72°C was extended to 10 minutes. To prevent evaporation, reaction mixtures were covered with 80μL of mineral oil.

For HPV 16 LCR primers, 100μL reactions were similar except for the $MgCl_2$ concentration which was determined to be optimal at 2.5mM. These reactions were also amplified for 35 cycles, consisting of 1 minute at 94°C, 2 minutes at 60°C and 2 minutes at 72°C, which was also extended to 10 minutes during the last cycle.

Each reaction series contained a number of clinical samples and three controls. These controls include no DNA, HPV negative DNA and HPV positive DNA. The 'no DNA' contained all reagents except the template DNA and served to monitor contamination of reaction components with exogenous HPV sequences. An HPV negative DNA was included to ensure specificity of the amplification product. The DNA

used was 'WINN DNA', which was demonstrated to be negative for HPV in all earlier experiments. 250ng was used in each PCR control reaction. An HPV positive control, 1000pg CaSki DNA, was included to ensure that the desired size amplification product was obtained and that proper reaction conditions were maintained. Caski is a cervical cancer cell line known to contain 50-500 copies of HPV 16 DNA.

Gel Electrophoresis of Amplified Samples

Following amplification of products, 20μL of reaction mixture was removed and added to 2μL of gel loading buffer (50% glycerol, 0.1% bromophenol blue). The amplification products were then separated by gel electrophoresis in a 1.5% agarose (Bishop) gel in 1X TAE (Tris-Acetate EDTA) containing 1μg per mL ethidium bromide. DNA fragments were viewed under a UV transilluminator. Initially, a molecular weight marker (ϕX-174 DNA digested with Hae III, Pharmacia) was used as well as the positive control as a reference for the appropriate sized DNA amplification product. Photographs were taken with a MP-4 polaroid camera through a red filter.

Analysis of Amplification Products and DNA Isolation and Extraction from Agarose Gels

Amplimers were purified through a column (Wizard PCR Preps DNA Purification System, Promega) if a single amplified product of the appropriate size was seen on the ethidium bromide stained gel. If there were multiple bands present, the rest of the

amplified product was separated by gel electrophoresis and the bands of interest were extracted with clean razor blades.

The DNA present in the excised bands was eluted using the Wizard DNA Purification System (Promega) and eluted in water. An ethanol precipitation was done overnight to concentrate the DNA.

Sequencing

Samples were sent to The Central Facility of the Institute for Molecular Biology and Biotechnology, McMaster University. If sequence data showed that more than one template was present in a sample, cloning was done to ascertain whether there was an infection with multiple genotypes. Samples were sequenced using Manos and HPV 16 type-specific primers, as previously described.

Cloning of PCR Product

HPV clinical samples were amplified again using Manos primers under similar conditions as previously described except Vent polymerase (New England Biolabs) was used instead of Taq polymerase. Vent polymerase produces >95% blunt ends whereas Taq polymerase generally produces 3' A overhangs. Using Vent polymerase eliminates the need for blunting the DNA before ligation.

HPV amplimers were excised from 1.5% agarose gels and placed in a 1.5mL Eppendorf tube. The DNA was purified using a GeneClean Kit (Bio 101 Inc.) and

reamplified. HPV amplimers were again excised and purified. Bluescript KS- vector was digested with EcoRV (Pharmacia) and an aliquot of the product was separated by electrophoresis (1.5% agarose) to ensure that linearization took place. One half of the HPV DNA was inserted into the Bluescript KS- digested with ECORV using T4 ligase (New England Biolabs), 1mM hexamine cobalt chloride (Sigma) and 1X ligase buffer (contains 1mM ATP, New England Biolabs). The ligation proceeded overnight at 13°C after which the enzyme was heat inactivated at 65°C for 10 minutes.

MAX Efficiency DH5-alpha competent cells (GIBCO, BRL) were used for transformations. Briefly, all of the ligation reaction (10μL) was added to the 50μL competent cells, mixed gently and incubated on ice for 30 minutes. A separate transformation reaction containing 1ng of Bluescript plasmid DNA was included as a positive control. The cells were heat shocked for 2 minutes at 42°C and placed at room temperature for 10 minutes. One millilitre of Luria-Bertani (LB) medium was added to the cells and the mixture was placed at 37°C for 45 minutes with shaking. The cells were resuspended in 400μL LB medium.

Different volumes of both control and experimental reactions were spread on LB agar plates containing 100 μg/mL ampicillin, 20 μg/mL X-gal (5-bromo-4-chloro-3 indolyl-β-D-galactoside) and 0.1mM IPTG (isopropyl-1-thio-β-D-galactoside) and incubated overnight at 37°C. Transformed colonies were selected based on their colour and inoculated into 10mL LB growth medium with 100 μg/mL ampicillin and incubated overnight at 37°C with continuous shaking. Plasmid DNA was isolated with QIAprep

Spin Miniprep Kits (Qiagen) and column purified by elution with water.

Approximately half the plasmid DNA (plus insert) was digested with XbaI (Pharmacia) for 3 hours at 37°C. Bluescript plasmid DNA without insert (10ng) was digested as a control. Digested DNA was seperated on ethidium bromide stained 1.5% agarose gels to detect differences in mobility.

Bluescript KS- is a 2.96 kb vector that has one XbaI site in the multiple cloning site. Therefore, a digestion of Bluescript with XbaI would result in a single band at 2.96 kb. The insert DNA is approximately 450bp in size. An XbaI digestion with the plasmid DNA, if the insert DNA was present, would result in a single band at approximately 3.5 kb. DNA from clones containing inserts of the correct size, determined with the Lambda marker (Lambda DNA digested with Hind III, Pharmacia), were stored at -20°C until DNA sequencing was performed.

Transformation was repeated using DH5α cells that were made competent using calcium chloride, according to standard protocols. Briefly, Escherichia coli cells are grown to log phase, cells are concentrated by centrifugation and resuspended in a solution containing calcium chloride and stored at -70°C. This exposure to calcium ions renders the cells capable of taking up DNA. Control and experimental reactions were spread on LB agar plates with 100 μg/mL ampicillin and without X-Gal or IPTG.

Hybridization Transfer Membranes for colony screening (New England Nuclear, Life Science Products) were placed on the agar plates containing transformants. The membranes were removed and placed on a small amount (1mL) of 0.1M NaOH on Saran

wrap for 2 minutes to lyse the cells. This step was repeated using fresh NaOH and Saran. Membranes were neutralized in the same manner with 1M Tris-HCl pH 7.5.

Membranes were then placed under UV light for 2.5 minutes in order to crosslink the DNA. Membranes were kept in hybridization buffer (100mM NaCl, 50mM Pipes, 50 mM Na_2HPO_4, 50mM $Na_2HPO_4 \cdot H_2O$, 1mM EDTA, 5% SDS).

The DNA probe, HPV insert DNA, was labelled with 50μCi of alpha-[^{32}P]-dCTP (3000 Ci/mmol) [New England Nuclear] using a T7 DNA polymerase labelling kit (T7 QuickPrime Kit, Pharmacia).

Membranes were placed in a hybridization chamber with 10mL hybridization buffer and probe (50μL) and rotated at 65°C overnight. Following hybridization, membranes were washed with 0.2X SSC/0.1% SDS for 45 minutes at 55°C. Autoradiography was performed at -70°C with Kodak XAR film overnight.

Colonies were picked based on hybridization results and plasmid DNA isolation and restriction digestions were performed as before. DNA was stored at -70°C until sequencing was performed.

Data Interpretation

DNA sequence homology analysis was performed using BLAST from GenBank at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). Sequence alignments were performed using ALIGN from Genestream Resource Center (http://www2.igh.cnrs.fr/bin/align-guess.cgi). Sequence results were compared to known

nucleotide sequences from the Human Papillomavirus Database on the Internet (http://hpv-web.lanl.gov/).

CHAPTER 1: AMPLICOR LINE BLOT ASSAY

INTRODUCTION

Our laboratory has previously examined clinical samples for evidence of HPV infection. The results described here relate to a subset of the clinical sample population which was discordant with respect to the data obtained with different tests designed to assess HPV infection status.

In particular, we have focussed the analysis on samples which had evidence of HPV as measured by a consensus PCR reaction (Manos *et al.*, 1989) and/or hybridization at low stringency with HPV nucleic acid probes but were discordant when assessed by an Amplicor line blot assay.

RESULTS

Sixteen samples obtained from University of Toronto students attending a health clinic for non-gynecological reasons were assessed for HPV infection status by three methodologies. This includes PCR using type-specific primers (6/11, 16, 18, 33) as well as L1 Manos consensus primers and E1 Gregoire consensus primers for any HPV. PCR reactions were assessed for HPV infection by visualization of an amplimer using gel

electrophoresis on ethidium bromide stained agarose gels. Secondly, aliquots from Manos consensus primer amplified reactions were evaluated for HPV by dot blot hybridization using a probe cocktail containing HPV 6, 11, 16, 18 and 33 DNA. Most recently, these samples were assessed by Amplicor strip analysis, which employs a combination of Manos consensus PCR (Manos *et al.*, 1989) and nonisotopic hybridization to discriminate amongst twenty-seven HPV genotypes. The summary of data obtained for each sample is presented in Table 2.

For each of the samples in Table 2, the L1 region PCR product was sequenced. The sequences obtained from each of these samples except sample #121 is shown in Figures 3 to 10. Each sample is aligned to its HPV type. Dots above the sequence represent a nucleotide change in the sequence. An N in the sequence, instead of one of the four nucleotides, denotes an inability to discriminate amongst the four nucleotides. A summary of the data with respect to HPV types is presented in Table 3.

The sequence from sample #121 indicated that more than one template existed, suggesting an HPV infection with multiple types. The PCR product from sample #121 was cloned into Bluescript and a number of clones were sequenced. Figure 11 shows the nucleotide changes for the seven clones containing HPV, specifically HPV CP6108. Clone numbers are indicated below the HPV CP6108 prototype sequence. A summary of sample #121 cloning data with respect to HPV type and homology to prototype is shown in Table 4. The common amino acid changes for the seven clones containing HPV CP6108 of sample #121 is presented in Figure 12.

TABLE 2: RESULTS OF HPV TESTING (DISCORDANT SAMPLES)

SAMPLE #	ETHIDIUM BROMIDE	DOT BLOT 55°	DOT BLOT 65°	AMPLICOR RESULTS	SAMPLE #	ETHIDIUM BROMIDE	DOT BLOT 55°	DOT BLOT 65°	AMPLICOR RESULTS
25	M+	+	+	-ve gel +ve	F107	M+	N.D.		-ve gel +ve
43	M+	+	+	-ve gel +ve	2975	M+	N.D.		-ve gel +ve
121	M+	+	+	-ve gel +ve	2764	M+, 16+	N.D.		-ve gel -ve ②
391	M+	+	-	-ve gel +ve	3242	M+	N.D.		-ve gel +ve ②
777	M+, G+, 16+	+	+	-ve gel +ve ①	3111	M+	-	+	-ve gel -ve
779	M+, G+	+	-	-ve gel +ve	1410	M+, 16+	+	+	16+, 51+
1468	M+, 16+	+	-	-ve gel +ve	3069	M+	N.D.		-ve gel -ve
2011	M+	+	+	-ve gel +ve	1378	M+, 16+, 6+/11+	N.D.		6+, 54+, MM8+ ②

M+ = positive by Manos primers (L1 consensus primers)
G+ = positive by Gregoire primers (E1 consensus primers)
D.B. = dot blot ; dot blot has 5 probe mixture of HPV 6, 11, 16, 18, 33

① = performed twice- once negative, once HPV 31 positive
② = performed twice with same result
N.D. = not done

FIGURE 3: L1 REGION SEQUENCES FOR HPV GENOTYPING (SAMPLES #25 AND #43)

Numbers indicate the nucleotide positions relative to the HPV prototype. Dots represent nucleotide changes between the sample sequence and the prototype sequence. An N in the sequence, instead of one of the four nucleotides, denotes an inability to discriminate amongst the four nucleotides. The underlined sequence is the primer. The percent homology to the prototype is given below the sequence.

SAMPLE # 25

25 TTTGTTGGTTTAATNAA- TGTTTGTTACTGTGGTGGATACTACTAAGAGTACTAATTTTACTATTTGTACCGCCTCCACTGCTGCAGCAGA
HPV62 [23]TTTGTTGGTTTAATGAACTGTTTGTTACTGTGGTGGATACTACCAGAAGTACTAATTTTACTATTTGTACCGCCTCCACTGCTGCAGCAGA

ATACAAGGCTACCAACTTTAGGGAATTTTTGCGACACACGGAGGAATTTGATTTGCAATTTATATTTCAATTGTGCAAAATACAGTTAAC
ATACACGGCTACCAACTTTAGGGAATTTTTGCGACACACGGAGGAATTTGATTTGCAATTTATATTTCAATTGTGCAAAATACAGTTAAC

CCCCGAAATCATGGCCTACCTGCATAATATGAACAAGGACCTTTTGGATGACTGGAACTTTGGGGTTTTACCTCCCCCTTCCACTAGTTT
CCCCGAAATTATGGCCTACCTGCATAATATGAACAAGGACCTTTTGGATGACTGGAACTTTGGGGTTTTACCTCCCCCTTCCACTAGTTT

AGATGAGACATATCACTATTTGCAGTCTCGGGCTATTACATGTCAAAAGGGGGCTGCTTCCCCGTCCCCCAAGGTGGACCCGTATGCGC
AGATGAGACATATCACTATTTCGAGTCTCGGGCTATTACATGTCAAA - GGGGGCTGCCTACCCGTCCC- - AAGGTGGACCCGTATGCGC

AAATGACATTTTGGACTGTGGATCTTAAGGACAAGTTGTCTACTGATTTGGATCAGTATCCCTGGGAAG
AAATGACATTTTGGACTGTGGATCTTAAGGACAAGTTGTCTACTGATTTGGATCAGT TTCCTTGGGTTG[449]

HOMOLOGY = 96.6%

SAMPLE # 43

43 CAAATCAGGGGAAAA- TTGTCTCGTAAATCAACAGTCCAAAATGATAATTTGGCATAGCGATCCTCCTTGGGCGGCGGGGCAGCAGCA
HPV 61 [714]CAAATCAGTGGAAAACTTGTCTCGTAAATCAACAGTCCAAAAGGATAACTTGGCATAGCGATCCTCCTTGGGCGGCGGGGCAGCAGCA

CCCTTCTGACATGTAATAGCTCTGGACTGCAAAAACCTATATGTGTCTTCTAGACTGGTAGAGGGTGGAGGTACCACACCAAAGTTCCA
CCCTTCAGACATGTAATAGCTCTGGACTGCAAAAACCTATATGTGTCTTCTAAACTGGTAGAGGGTGGTGGTACCACACCAAAGTTCCA

GTCATCCAACAAGGCTTTATTCATATTATGTAGGTAGGCCATAATTTCAGGGGTTAAATGTATTTTACATAACTGAAAAATGAATTGCA
GTCATCCAACAAGGCCTTATTCATATTATGTAGGTAGGCCATAATTTCAGGGGTTAAATGTATTTTACATAACTGAAAAATAAATGGCA

AATCAAACTCTTCTGTATGGCGCAAATATTCCCTAAAGCTCGTGGCTTTATATTCAGATACAGGGGGGGATGTAGCAGTACAAATGGTT
AATCAAACTCCTCTGTATGGCGCAAATATTCCCTAAAGCTTGTGGCTTTATATTCAGATACAGGGGGGGATGTAGCAGTACAAATGGTT

ACATTAGTACTGCGGGTGGTATCCACAACGGTTACAAACAATTCATTAAACCAACAAATACCATTNTTATGACCCNGNNG
AAATTAGTACTGCGGGTGGTATCCACAACGGTTACAAACAATTCATTAAACCAACAAATACCATTGTTGTGGCCCTGGGC[4712]

HOMOLOGY = 96.9%

FIGURE 4: L1 REGION SEQUENCES FOR HPV GENOTYPING (SAMPLES #391 AND #777)

SAMPLE # 391

391 TTTAATAGAATTGTTTGTAACCGTTGTGGATACCACCCGCAGTACTAATGTAACCATTTGTACTGCTACATCCCCCCCTGTATCTGAATA
HPV 61 6742TTTAAT- GAATTGTTTGTAACCGTTGTGGATACCACCCGCAGTACTAATTTAACCATTTGTACTGCTACATCCCCCCCTGTATCTGAATA

TAAAGCCACGAGCTTTAGGGAATATTTGCGCCATACAGAAGAGTTTGATTTGCAATTCATTTTTCAGTTATGTAAAATACATTTAACCCC
TAAAGCCACAAGCTTTAGGGAATATTTGCGCCATACAGAAGAGTTTGATTTGCAATTCATTTTTCAGTTATGTAAAATACATTTAACCCC

TGAAATTATGGCCTACCTACATAATATGAATAAAGCCTTGTTGGATGACTGGAACTTTGGTGTGGTACCTCCACCCTCTACCAGTCTAGA
TGAAATTATGGCCTACCTACATAATATGAATAAGGCCTTGTTGGATGACTGGAACTTTGGTGTGGTACCACCACCCTCTACCAGTTTAGA

AGACACATATAGGTTTTTGCAGTCCAGAGCTATTACATGTCAGAAGGGTGCTGCTGCCCCGCCGCCCAAGGAGGATCGCTATGCCAAAT
AGACACATATAGGTTTTTGCAGTCCAGAGCTATTACATGTCTGAAGGGTGCTGCTGCCCCGCCGCCCAAGGAGGATCGCTATGCCAAGT

TATCATTTTGGACTGTTGATTTACGAGACAAGTTTTCCACTGATTTGGATCAGTTTCCCCTGGGNAG
TATCCTTTTGGACTGTTGATTTACGAGACAAGTTTTCCACTGATTTGGATCAGTTTCCTTTGGGGCG7146

HOMOLOGY = 97.8%

SAMPLE # 777

777 GGGGCAATCAGTTATTTGTTACTGTGGTAGATACCACACGTAGTACCAATATGTCTGTGTGTGCTGCAATTGCAAACAGTGATACTACAT
HPV 31 6528GGGGCAATCAGTTATTTGTTACTGTGGTAGATACCACACGTAGTACCAATATGTCTGTGTGTGCTGCAATTGCAAACAGTGATACTACAT

TTAAAAGTAGTAATTTTAAAGAGTATTTAAGACATGGTGAGGAATTTGATTTACAATTTATATTTCAGTTATGCAAAATAACATTGTCTG
TTAAAAGTAGTAATTTTAAAGAGTATTTAAGACATGGTGAGGAATTTGATTTACAATTTATATTTCAGTTATGCAAAATAACATTATCTG

CAGACATAATGACATATATTCACAGTATGAATCCTGCTATTTTGGAANATTGGAATTTTGGATTGACCACACCTCCCTCAGGTTCTTTAG
CAGACATAATGACATATATTCACAGTATGAATCCTGCTATTTTGGAAGATTGGAATTTTGGATTGACCACACCTCCCTCAGGTTCTTTAG

AGGATACCTATAGGTTTGTAACCTCACAGGCCATTACATGTCAAAAAACTGCCCCCCAAAAGCCCAAGGAANATCCATTTAAAGATTAT
AGGATACCTATAGGTTTGTAACCTCACAGGCCATTACATGTCAAAAAACTGCCCCCCAAAAGCCCAAGGAAGATCCATTTAAAGATTAT

GTATTTTGGGAGGTTAATTTAAAANAAAAGTTTTCTGCANATTTAGATCAGTTTCCCCTGGGAAG
GTATTTTGGGAGGTTAATTTAAAAGAAAAGTTTTCTGCAGATTTAGATCAGTTTCCACTGGGTCG9

HOMOLOGY = 98.8%

Refer to Figure 3 for legend.

FIGURE 5: L1 REGION SEQUENCES FOR HPV GENOTYPING (SAMPLES #779 AND #1468)

SAMPLE # 779

HOMOLOGY = 98.7%

SAMPLE # 1468

HOMOLOGY = 86.9%

Refer to Figure 3 for legend.

FIGURE 6: L1 REGION SEQUENCES FOR HPV GENOTYPING (SAMPLES #2975 AND #F107)

SAMPLE # 2975

2975 AAATCAATGCNAAAATTGTCTCGTAAATCAACAGTCCAAAAGGATAACTTGGCATACCGATCCTCCTTGGGCGGCGGGGCAGCAGCAC

HPV 61 ^{7145}AAATCAGTGGAAAACTTGTCTCGTAAATCAACAGTCCAAAAGGATAACTTGGCATAGCGATCCTCCTTGGGCGGCGGGGCAGCAGCAC

CCTTCTGACATGTAATAGCTCTGGACTGCAAAAACCTATATGTGTCTTCTAAACTGGTAGAGGGTGGTGGTACCACACCAAAGTTCCAG

CCTTCAGACATGTAATAGCTCTGGACTGCAAAAACCTATATGTGTCTTCTAAACTGGTAGAGGGTGGTGGTACCACACCAAAGTTCCAG

TCATCCAACAAGGCCTTATTCATATTATGTAGGTAGGCCATAATTTCAGGGGTTAAATGTATTTTACATAACTGAAAAATAAATTGCAA

TCATCCAACAAGGCCTTATTCATATTATGTAGGTAGGCCATAATTTCAGGGGTTAAATGTATTTTACATAACTGAAAAATAAATGGCAA

ATCAAACTCCTCTGTATGGCGCAAATATTCCCTAAAGCTTGTGGCTTTATATTCAGATACAGGGGGGGATGTAGCAGTACAAATGGTTA

ATCAAACTCCTCTGTATGGCGCAAATATTCCCTAAAGCTTGTGGCTTTATATTCAGATACAGGGGGGGATGTAGCAGTACAAATGGTTA

AATTAGTACTGCGGGTGGTATCCACAACGGTTACAAACAATTCATTAAACCAACAAATACCATTGTTATGACCCTGTNG

AATTAGTACTGCGGGTGGTATCCACAACGGTTACAAACAATTCATTAAACCAACAAATACCATTGTTGTGGCCCTGGGC^{6712}

HOMOLOGY = 98.3%

SAMPLE # F107

F107 CAACTTGTNCTTAAGATCCACAGTCCANAATGTCATTTGCNCATACGGGTCCACCTTGGGGGACGGGGAAGCANCCCCCTTTTGACATG

HPV 62 ^{417}CAACTTGTCCTTAAGATCCACAGTCCAAAATGTCATTTGCGCATACGGGTCCACCTTGG - -GACGGGTAGGCAGCCCCCTTT -GACATG

CAATAGCCCGAAACTGCAAATAGTGATATGTCTCATCTAAACTAGTGGAAGGGGGAGGTAAAACCCCAAAGTTCCANTCATCCAAAAG

TAATAGCCCGAGACTCGAAATAGTGATATGTCTCATCTAAACTAGTGGAAGGGGGAGGTAAAACCCCAAAGTTCCAGTCATCCAAAAG

GTCCTTGTTCATATTATGCANGTAGGCCATGATTTCGGGGGTCAACTGTATTTTGCACAATTGAAATATAAATTGCAAATCAAATTCCTC

GTCCTTGTTCATATTATGCAGGTAGGCCATAATTTCGGGGGTTAACTGTATTTTGCACAATTGAAATATAAATTGCAAATCAAATTCCTC

CGTGTGTCGCAAAAATTCCCTAAAGTTGGTGGCCTTGTATTCTGCTGCANTAGTGGAGGCGGTACAAATAGTAAAATTAGTACT CCTAG

CGTGTGTCGCAAAAATTCCCTAAAGTTGGTAGCCGTGTATTCTGCTGCAGCAGTGGAGGCGGTACAAATAGTAAAATTAGTACTTCTGG

TAGTATCCACCACAGTAACAAACAGGTCATTAAACCAACAAATACCATTATTATGNCCCTGNGC

TAGTATCCACCACAGTAACAAACAGTTCATTAAACCAACAAATACCATTATTATGACCCTGTGC^{1}

HOMOLOGY = 94.0%

Refer to Figure 3 for legend.

FIGURE 7: L1 REGION SEQUENCES FOR HPV GENOTYPING (SAMPLES #3111 AND #2011)

SAMPLE # 3111

3111 TTGTCTCGTAAGNNAACCGTCCAAAATGATAATTTGGCATAGCGATCCTCCTTGGGCGGCGGGGCAGCAGCACCCTTCTGACATGTAA
HPV 61 7139TTGTCTCGTAAATCAACAGTCCAAAAGGATAACTTGGCATAGCGATCCTCCTTGGGCGGCGGGGCAGCAGCACCCTTCAGACATGTAA

TAGCTCTGGACTGCAAAAACCTATATGTGTCTTCTAGACTGNGTAGAGNGGTGGGAGGTACCCACACCAAAGTTCCAGNCATCCAACA
TAGCTCTGGACTGCAAAAACCTATATGTGTCTTCTAAACTG - GTAGAG -GGTGGT - GGTACC -ACACCAAAGTTCCAG TCATCCAACA

AGGCTTTATTCATATTATGTAGGTAGNGCCATAATTTCAGGNGGTTAAATGTATTTTACATAACTGAAAAAATGAATTGCAAATCAAAC
AGGCCTTATTCATATTATGTAGGTAG -GCCATAATTTCAGG - GGTTAAATGTATTTTACATAACTGAAAAA -TAAATGGCAAATCAAAC

TCTTCTGTATGGCGCAAATATTCCCTAAAGCTCGTGGGCTTTATATTCAGATACAGGGGGGGGATGTAGCAGTACAAAATGGGTTACAT
TCCTCTGTATGGCGCAAATATTCCCTAAAGCTTGTGG- CTTTATATTCAGATACAGGGGGGG - ATGTAGCAGTACAAAT - - GGTTAAAT

TAGTACTGCGGGTGGGTATCCACAACGGTTACAAACAATTCATTAAACCAACAAATACCATTGTTTATTGACCCCGGGG
TAGTACTGCGGGTGG - TATCCACAACGGTTACAAACAATTCATTAAACCAACAAATACCATTGTTG - -TGGCCCTGGGC6712

HOMOLOGY = 92.9%

SAMPLE # 2011

2011 GACAAACGTTCCTTAAGATCCACATCCCAAAAGGATAACTTATCATATGGATCCTTTTTAGGTTCTGGGGCAGCAGTGCCCTTTTGACAT
CP6108 422GACAAACGTTCCTTAAGATCCACATCCCAAAAGGATAACTTATCATATGGATCCTTTTTAGGTTCTGGGGCAGCAGTGCCCTTTTGACAT

GTAATGGCCCGAGAGGTAAGAAAGCGATAGGTATCATCCAAACTAGTGGAGGGAGGGGGAATGACACCAAAGTTCCATTCATCTAACA
GTAATGGCCCGAGAGGTAAGAAAGCGATAGGTATCATCCAAACTAGTGGAGGGAGGGGGAATGACACCAAAGTTCCATTCATCTAACA

ATGTGTCATTCATATTGTGTAAATAGGACATTATCTCAGGCGTTAGGTGTATCTTACATAGTTGGAATATAAACTGTAGGTCATATTCCT
ATGTGTCATTCATATCGTGTAAATAGGACATTATCTCAGGCGTTAGGTGTATCTTACATAGTTGGAATATAAACTGTAGGTCATATTCCT

CAGTGTGTCTTAAATATTCCTTAAAGCGTGTAGAACTGTATTCTGTGGCAGACTGGGAAGCAGCACAAATGGTAAGGTTGGTACTACGG
CAGTGTGTCTTAAATATTCCTTAAAGCGTGTAGAACTGTATTCTGTGGCAGACTGGGAAGCAGCACAAATGGTAAGGTTGGTACTACGG

GTGGTATCTACCACAGTAACAAACAACTCATTAAACCAACAAATACCATTATTATGNCCCTGTNGC
GTGGTATCTACCACAGTAACAAACAACTCATTAAACCAACAAATACCATTATTATGACCCTGT- GC1

HOMOLOGY = 99.8% Refer to Figure 3 for legend.

FIGURE 8: L1 REGION SEQUENCES FOR HPV GENOTYPING (SAMPLES #3069 AND #1378)

SAMPLE # 3069

```
3069      ACGTTCCTTAAGATCCACATCCCAAAAGGATAACTTATCATATGGATCCTTTTTAGGTTCTGGGGCAGCAGTGCCCTTTTGACATGTAAT
CP 6108  417ACGTTCCTTAAGATCCACATCCCAAAAGGATAACTTATCATATGGATCCTTTTTAGGTTCTGGGGCAGCAGTGCCCTTTTGACATGTAAT

          GGCCCGAGAGGTAAGAAAGCGATAGGTATCATCCAAACTAGTGGAGGGAGGGGGAATGACACCAAAGTTCCATTCATCTAACAATGTG
          GGCCCGAGAGGTAAGAAAGCGATAGGTATCATCCAAACTAGTGGAGGGAGGGGGAATGACACCAAAGTTCCATTCATCTAACAATGTG
                    •
          TCATTCATATTGTGTAAATAGGACATTATCTCAGGCGTTAGGTGTATCTTACATAGTTGGAATATAAACTGTAGGTCATATTCCTCAGTG
          TCATTCATATCGTGTAAATAGGACATTATCTCAGGCGTTAGGTGTATCTTACATAGTTGGAATATAAACTGTAGGTCATATTCCTCAGTG
                                                                                        •
          TGTCTTAAATATTCCTTAAAGCGTGTAGAACTGTATTCTGTGGCAGACTGGGAAGCAGCACAAATGGTAAGGGTTGGTACTACGGGTGG
          TGTCTTAAATATTCCTTAAAGCGTGTAGAACTGTATTCTGTGGCAGACTGGGAAGCAGCACAAATGGTAAGG - TTGGTACTACGGGTGG
          •
          GTATCTACCACAGTAACAAACAACTCATTAAACCAACAAATACCATTNTTATGACCCCGGNGC
           - TATCTACCACAGTAACAAACAACTCATTAAACCAACAAATACCATTATTATGACCCTGT - GC
```

HOMOLOGY = 99.2%

SAMPLE # 1378

```
                                                                        •                ••
1378      GGGGTAATCAACTGTTTGTTACTGTGGTAGATACCACACGCAGTACCAACATGACNTTATGTGCATCCGCNACTACATCTTCCACATAC
HPV 6b  4750GGGGTAATCAACTGTTTGTTACTGTGGTAGATACCACACGCAGTACCAACATGACATTATGTGCATCCGTAACTACATCTTCCACATAC
                                        •                                •                 • •          •
          ACCAATTCTGATTATAAAGAGTACATGCGNCATGTGGAAGAGTATGATTTACAATTTATNTTTCAATTATGTANCNTTACATTGACTGC
          ACCAATTCTGATTATAAAGAGTACATGCGTCATGTGGAAGAGTATGATTTACAATTTAT TTTTCAATTATGTAGCATTACATTGTCTGC
                                                    •                                                •
          TGAAGTAATGGCCTATATTCACACAATGAATCCCTCTGTNTTGGAAGACTGGAACTTTGGGTTATCGCCTCCCCCAAATGGNACATTA
          TGAAGTAATGGCCTATATTCACACAATGAATCCCTCTGTTTTGGAAGACTGGAACTTTGGGTTATCGCCTCCCCCAAATGG TACATTA
                                                                  •
          GAAGATACCTATAGGTATGTGCAGTCACAGGCCATTACCTGTCAAAAGNCCACTCCTGAAAAGGAAAAGCCAGATCCCTATAAGAAC
          GAAGATACCTATAGGTATGTGCAGTCACAGGCCATTACCTGTCAAAAGCCCACTCCTGAAAAGGAAAAGCCAGATCCCTATAAGAAC
              ••  •          ••                ••••  •            •
          CTTATNTTCTGGGAGGGATAATTTAAAAGANGGTTTGTCTAGTGAATGGGATCAAGTATCCCCTNNGGNN
          CTTAGTTTTTGGGAGGT - TAATTTAAAAGAAAAGTTTTCTAGTGAATTGGATCA - GTATCCTTTGGGACG7170
```

HOMOLOGY = 94.5%

Refer to Figure 3 for legend.

FIGURE 9: L1 REGION SEQUENCES FOR HPV GENOTYPING (SAMPLE #3242)

SAMPLE # 3242

Refer to Figure 3 for legend.

FIGURE 10: L1 REGION SEQUENCES FOR HPV GENOTYPING (SAMPLES #2764 AND #1410

SAMPLE # 2764

2764 CAATCAATTGTTTTTAACAGTTGTAGATACTACTCGCAGCACCAATCTTTCTGTGTGTGCTTCTACTACGTCTTCTATTCCTAATGTATAC

HPV 59 [6583]CAATCAATTGTTTTTAACAGTTGTAGATACTACTCGCAGCACCAATCTTTCTGTGTGTGCTTCTACTACTTCTTCTATTCCTAATGTATAC

ACACCTACCAGTTTTAAAGAATATGCCAGACATGTGGAGGAATTTGATTTGCAGTTTATATTTCAACTGTGTAAAATAACATTAACTACA
ACACCTACCAGTTTTAAAGAATATGCCAGACATGTGGAGGAATTTGATTTGCAGTTTATATTTCAACTGTGTAAAATAACATTAACTACA

GAGGTAATGTCATACATTCATAATATGAATACCACTATTTTGGAGGATTGGAATTTTGGTGTTACACCACCTCCTACTGCTAGTTTAGTT
GAGGTAATGTCATACATTCATAATATGAATACCACTATTTTGGAGGATTGGAATTTTGGTGTTACACCACCTCCTACTGCTAGTTTAGTT

GACACATACCGTTTTGTTCAATCTGCTGCTGTAACTTGTCAAAAGGACACCGCACCGCCAGTTAAACAGGACCCTTATGACAAACTAAA
GACACATACCGTTTTGTTCAATCTGCTGCTGTAACTTGT CAAAAGGACACCGCACCGCCAGTTAAACAGGACCCTTATGACAAACTAAA

GTTTTGGACTGTAGATCTTAAGGAAAGGTTTTCTGCAGATCTTGATCAGTTTCCCCTTGGGACG
GTTTTGGCCTGTAGATCTTAAGGAAAGGTTTTCTGCAGATCTTGATCAGTTTCC T- TTGGGACG[7105]

HOMOLOGY = 99.5%

SAMPLE # 1410

1410 TCTGCAGAAA- CTTTTCCTTTAAATTTACTTCCCAAAAAGTGTATTTTTTAAGGGGATCTTCTTTAGGTGCTGGAGGTGTATGTTTTTGAC

HPV 16 [7016]TCTGCAGAAAACTTTTCCTTTAAATTTACTTCCCAAAAAGTGTATTTTTTAAGGGGATCTTCTTTAGGTGCTGGAGGTGTATGTTTTTGAC

VARIANT

AAGCAATTGCCTGGGATGTTACAAACCTATAAGTATCTTCTAGTGTGCCTCCTGGGGGGGGTTGTAGACCAAAATTCCAGTCCTCCAAA
AAGCAATTGCCTGGGATGTTACAAACCTATAAGTATCTTCTAGTGTGCCTCCTGGGGGGGGTTGTAGACCAAAATTCCAGTCCTCCAAA

ATAGTGGAATTCATAGAATGTATGTATGTCATAACGTCTGCAGTTAAGGTTATTTTGCACAGTTGAAAAATAAACTGTAAATCATATTC
ATAGTGGAATTCATAGAATGTATGTATGTCATAACGTCTGCAGTTAAGGTTATTTTGCACAGTTGAAAAATAAACTGTAAATCATATTC

CTCCCCATGTCGTAGGTACTCCTTAAAGTTAGTATTTTTATATGTAGTTTCTGAAGTAGATATGGCAGCACATAATGACATATTTGTACT
CTCCCCATGTCGTAGGTACTCCTTAAAGTTAGTATTTTTATATGTAGTTTCTGAAGTAGATATGGCAGCACATAATGACATATTTGTACT

GCGTGTAGTATCAACAACAGTAACAAATAGTTGGTTACCCCAACAAATGCCATTATTATGACCCTGNGC
GCGTGTAGTATCAACAACAGTAACAAATAGTTGGTTACCCCAACAAATGCCATTATTGTGGCCCTGACG[6583]

HOMOLOGY = 99.8%

Refer to Figure 3 for legend.

TABLE 3: SEQUENCING DATA FOR DISCORDANT SAMPLES

SAMPLE NUMBER	SEQUENCE OF L1 REGION
25	HPV 62 96.6% homology
43	HPV 61 96.9% homology
121	mixed sequence → cloned; see Table 4
391	HPV 61 97.8% homology
777	HPV 31 98.8% homology
779	HPV CP8304 98.7% homology
1468	HPV 61 86.9% homology
2011	HPV CP6108 99.8% homology
F107	HPV 62 94.0% homology
2975	HPV 61 98.3% homology
2764	HPV 59 99.5% homology
3242	HPV XS4 97.3% homology
3111	HPV 61 92.9% homology
1410	HPV 16 variant 99.8% homology
3069	HPV CP6108 99.2% homology
1378	HPV 6 94.5% homology

FIGURE 11: NUCLEOTIDE CHANGES OF CLONES FOR SAMPLE #121 COMPARED TO HPV CP6108

#7 G inserted between position 43 and 44
#1 T inserted between position 387 and 388
#1 C inserted between position 233 and 234
#6 T deleted in position 417
#6 T inserted between position 349 and 350

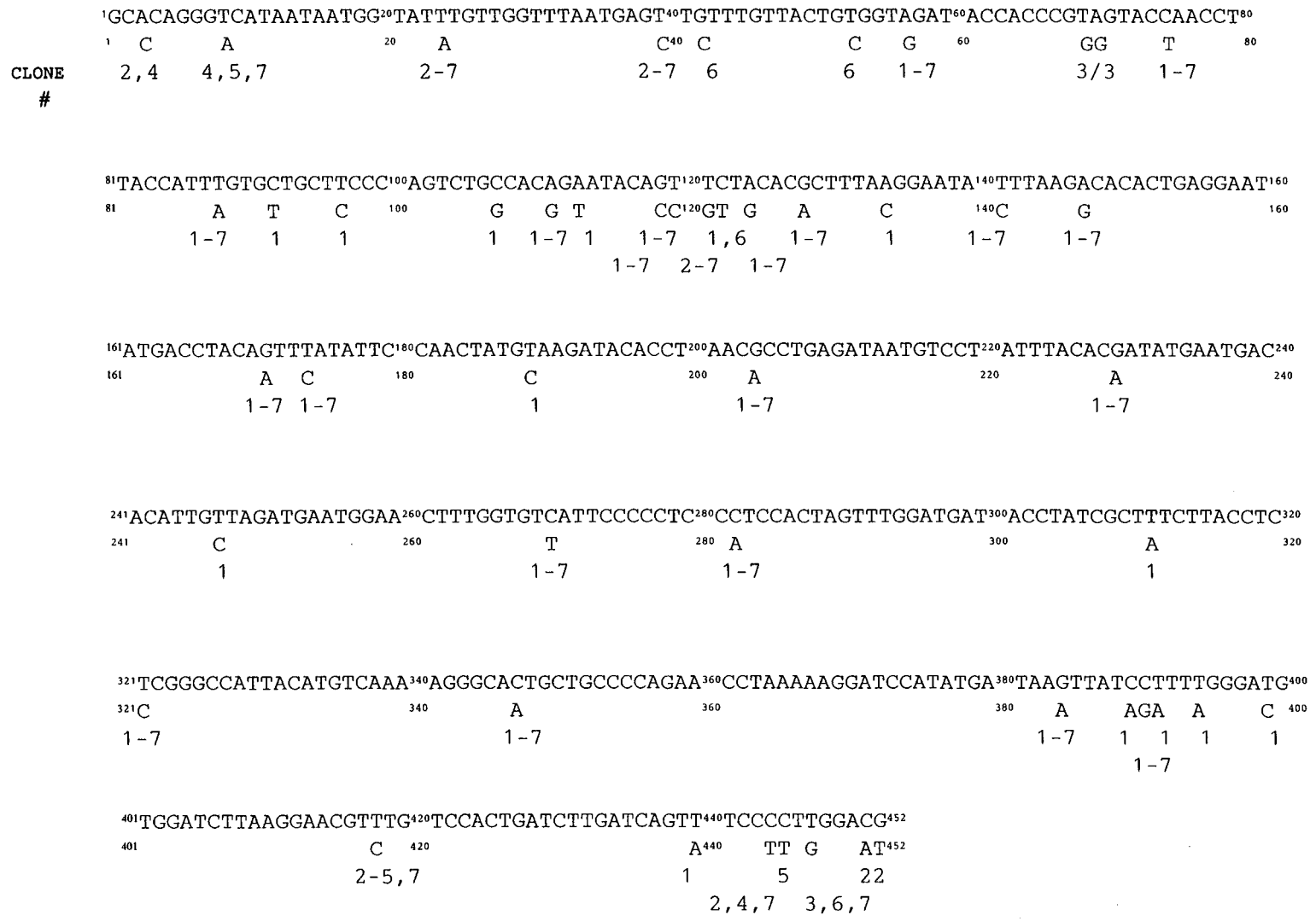
1GCACAGGGTCATAATAATGG20TATTTGTTGGTTTAATGAGT40TGTTTGTTACTGTGGTAGAT60ACCACCCGTAGTACCAACCT80
CLONE #
2,4 4,5,7 2-7 2-7 6 6 1-7 3/3 1-7
81TACCATTTGTGCTGCTTCCC100AGTCTGCCACAGAATACAGT120TCTACACGCTTTAAGGAATA140TTTAAGACACACTGAGGAAT160
1-7 1 1 1 1-7 1 1-7 1-7 1,6 1-7 1 1-7 1-7
1-7 2-7 1-7
161ATGACCTACAGTTTATATTC180CAACTATGTAAGATACACCT200AACGCCTGAGATAATGTCCT220ATTTACACGATATGAATGAC240
1-7 1-7 1 1-7 1-7
241ACATTGTTAGATGAATGGAA260CTTTGGTGTCATTCCCCCTC280CCTCCACTAGTTTGGATGAT300ACCTATCGCTTTCTTACCTC320
1 1-7 1-7 1
321TCGGGCCATTACATGTCAAA340AGGGCACTGCTGCCCCAGAA360CCTAAAAAGGATCCATATGA380TAAGTTATCCTTTTGGGATG400
1-7 1-7 1-7 1 1 1 1
1-7
401TGGATCTTAAGGAACGTTTG420TCCACTGATCTTGATCAGTT440TCCCCTTGGACG452
2-5,7 1 5 22
2,4,7 3,6,7

TABLE 4: CLONING RESULTS FROM SAMPLE #121

CLONE NUMBER	HPV GENOTYPE AND SEQUENCE OF L1 REGION
1	HPV CP6108 84.8% homology
2	HPV CP6108 94.3% homology
3	HPV CP6108 93.7% homology
4	HPV CP6108 94.2% homology
5	HPV CP6108 94.3% homology
6	HPV CP6108 93.0% homology
7	HPV CP6108 93.9% homology

Note: Independent colonies from one PCR reaction
7/17 sequences submitted

FIGURE 12: COMMON AMINO ACID CHANGES OF CLONES FOR SAMPLE #121 WITH HPV CP6108

ALA = alanine
ARG = arginine
ASN = asparagine
ASP = aspartic acid
CYS = cysteine
GLN = glutamine
HIS = histidine
LYS = lysine
PHE = phenylalanine
PRO = proline
SER = serine
THR = threonine
TYR = tyrosine
VAL = valine

```
1GCA CAG GGT CAT AAT AAT GG20T ATT TGT TGG TTT AAT GAG T40TG TTT GTT ACT GTG GTA GAT60 ACC ACC CGT
                                                                                  G
                                                                                 VAL

AGT ACC AAC CT80T ACC ATT TGT GCT GCT TCC C100AG TCT GCC ACA GAA TAC AGT 120TCT ACA CGC TTT AAG GAA
      T                   A                                G          CC       G    A
     THR                 CYS                             THR        SER       THR ARG
                          ↓                                          ↓         ↓   ↓
                         SER                                        THR       ALA HIS

TA140T TTA AGA CAC ACT GAG GAA T160AT GAC CTA CAG TTT ATA TTC180 CAA CTA TGT AAG ATA CAC CT200A ACG
    C          G                                A   C                                          A
  TYR         HIS                             GLN PHE                                        THR
               ↓
              ARG

CCT GAG ATA ATG TCC T220 AT TTA CAC GAT ATG AAT GAC240 ACA TTG TTA GAT GAA TGG AA260C TTT GGT GTC
                                   A                                                            T
                                  ASP                                                          VAL
                                   ↓
                                  ASN

ATT CCC CCT C280CC TCC ACT AGT TTG GAT GAT300 ACC TAT CGC TTT CTT ACC TC320T CGG GCC ATT ACA TGT
               A                                                          C
              PRO                                                       SER
               ↓
              HIS

CAA A340AG GGC ACT GCT GCC CCA GAA360 CCT AAA AAG GAT CCA TAT GA380T AAG TTA TCC TTT TGG GAT G400TG
              A                                                      A       G
             THR                                                    LYS    SER
              ↓
             ASN

GAT CTT AAG GAA CGT TTG 420TCC ACT GAT CTT GAT CAG TT440T CCC CTT GGA CG452
```

DISCUSSION

Many studies have looked at HPV type-specific prevalence in low and high grade abnormalities of the cervical epithelium preceding cervical cancer as well as in cervical carcinomas and the normal cervix. Various nucleic acid detection assays have been used for the detection of HPV DNA from clinical samples. The methods that identify and distinguish several HPV genotypes, Southern blot and dot blot of PCR product, are labour intensive and time consuming for routine clinical testing or for large population studies. PCR has proven to be sensitive and effective in identifying HPV DNA. However, the lack of methods to discriminate among a large number of HPV genotypes with respect to particular HPV types has made studies of disease association with clinical pathology difficult. Therefore, a new method or assay to type HPV infections, particularly the mucosotropic subset of HPVs, in a short period of time, is of interest to many researchers and clinical laboratories.

Roche Molecular Systems has recently developed a strip-based detection system which discriminates among twenty-seven HPV genotypes, both cancer and non-cancer associated types. The line blot assay combines consensus Manos PCR (Manos *et al.*, 1989) with nonisotopic detection of amplified DNA from the L1 region. The use of PCR in this method increases the sensitivity of HPV detection. This technique is easier to perform than Southern blot or other forms of hybridization analysis for HPV DNA

detection and may allow for quick and accurate typing. Figure 13 is a schematic representation of the Amplicor line blot genotyping assay.

In this laboratory, we have reassessed study samples found to be HPV positive by previous detection methods with this Amplicor HPV Genotyping Kit. Most of these agreed with previous results. A subset of samples was unexpectedly negative for HPV in the line blot assay. Aliquots of PCR reaction which were Amplicor strip negative were also examined on ethidium bromide stained gels to assess whether Manos consensus 450bp amplimers could be detected. In the first part of this study, part of the L1 gene of a number of HPV positive samples was sequenced. The sequence data results are summarized in Table 3.

Sequence Data

Most of the samples sequenced reveal homologies suggestive of five HPV 61 genotypes [samples #43 (Figure 3), #391 (Figure 4), #1468 (Figure 5), #2975 (Figure 6) and #3111 (Figure 7)], two HPV 62 [samples #25 (Figure 3) and #F107 (Figure 6)], two HPV CP6108 [samples #2011 (Figure 7) and #3069 (Figure 8)] and one HPV CP8304 [sample #779 (figure 5)]. All of these genotypes are not represented on the Amplicor strips. These last two HPV types were first identified by Peyton *et al.* (1994) in the New Mexico triethnic population (Native Americans, Hispanics and non-Hispanic whites). Several of the samples, in Table 2, have ambiguous nucleotides in the sequences, which indicates that the nucleotide could not be distinguished at that position and this artificially

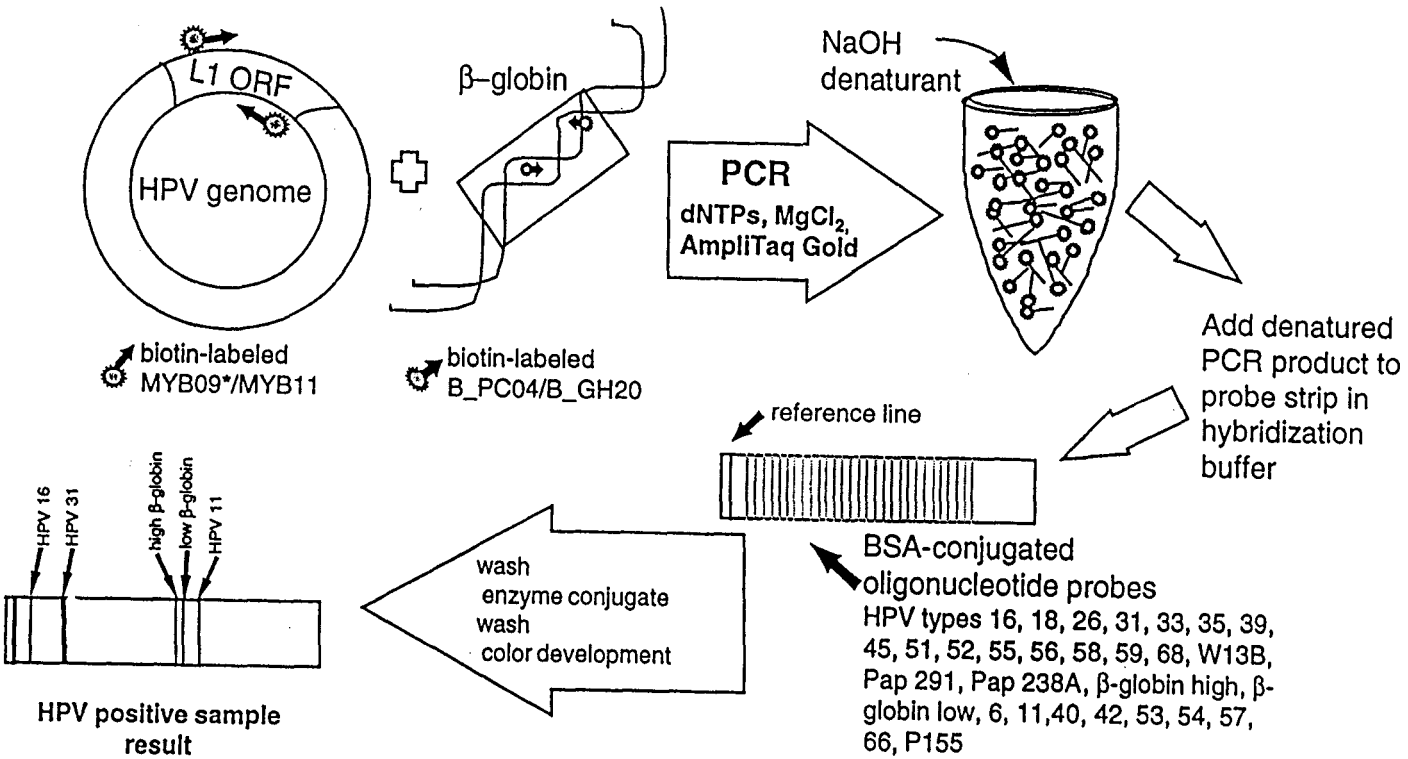

FIGURE 13: AMPLICOR LINE BLOT ASSAY

The drawing is a representation of a hypothetical HPV mixed infection with genotypes 11, 16 and 31. HPV DNA is amplified using PCR by L1 biotin-labeled consensus primer pairs (MYB09/MYB11). Biotin-labeled β-globin primers (B_PC04/B_GH20) are used to assess sample adequacy. The PCR product was denatured and the HPV genotyping strips are place in hybridization buffer along with denatured biotinylated PCR product. Strips are washed; enzyme conjugate is added and strips are washed again to remove the unbound conjugate. Colour development takes place with addition of substrates (Gravitt *et al.*, 1998).

decreases the homology to the prototype. In particular, sample #1468 is probably HPV 61 but due to the sequence ambiguity, had a homology of 86.9% (see Figure 5). Eliminating ambiguous bases from consideration increases the homology to 94.0%. As previously stated, a genotype is defined as an HPV that has <90% homology to the closest related HPV in the L1 region of the genome. A subtype, on the other hand, shares 90-98% homology and a variant has >98% homology. Consequently, sample #1468 with the N's in the sequence would be considered a new genotype. On the other hand, without the N's, sample #1468 would be a subtype of HPV 61. For the other samples, eliminating the N's, the homologies also increase, but only slightly. Sample #25 would be 96.8% homologous to HPV 62, #3111 would have a homology of 94.6% to HPV 61, #F107, a homology of 95.5% to HPV 62, #779, a homology of 99.2% to HPV CP8304 and #2975 would be 98.6% homologous to HPV 61 (Table 3). These samples are less important to draw attention to because samples #25, #F107 and #3111 remain subtypes and samples #779 and #2975 remain variants to their respective types.

Sample #3242 was found to be a new HPV genotype, HPV XS4. Sequence results show 97.3% homology to a partial L1 (261bp) sequence that was recently added into the HPV Database. However, it has not yet been published (Berkhout, R.J. and ter Schegget, J., http://hpv-web.lanl.gov/) in a peer reviewed journal. The next closest HPV genotype in homology is HPV MM8 but there is a significant decrease in homology to 82.5%. Both of these sequences are in Figure 9. The L1 sequence of sample #1410 demonstrates 99.8% homology to an unpublished HPV 16 variant (length = 7905bp)

which was found in a Caski cervical cancer cell line (http://hpv-web.lanl.gov/). This sequence is shown in Figure 10.

Initial sequence data only revealed two samples (#777 and #2764) which should have been detected by the strips but were not. Sample #777 is 98.8% homologous to HPV 31 (99.8% ignoring the minor sequence ambiguity) and #2764 is 99.5% homologous to HPV 59. When the Amplicor strips were repeated on these two samples, sample #777 was shown to be HPV 31 positive which is concordant with sequence data however sample #2764 remained strip negative (Table 2). The sequence for sample #777 can be seen in Figure 4. The reason for different results on repeat analysis for this sample is most likely due to a problem with the strip or a co-infection in which different HPV types are amplified at different efficiencies between reactions.

Three of the samples we studied were discordant between consensus Manos PCR results and Amplicor results. Samples #2764, #3069 and #3111 were all Manos positive by examination of ethidium bromide stained amplimers but failed to show an amplimer when biotinylated Manos amplification products were electrophoretically separated on an agarose gel. We reamplified these three samples to sequence the amplimers with the Manos consensus primers. The ethidium bromide stained gel showed a strong Manos band for each sample. These bands were consistent with the size of the control amplimer, a cervical cancer cell line which contains HPV 16. Sequencing data revealed that all of these samples contained HPV. Sample #2764 is 99.8% homologous to HPV 59, #3069 is 99.2% homologous to HPV CP6108 and sample #3111 is 92.9% homologous to HPV 61

(Table 3).

We had initially hypothesized that the patient samples which are ethidium bromide and/or dot blot positive but were Amplicor strip negative might be HPV genotypes which were not represented on the strips. Samples #3069 and #3111 contain HPV genotypes which are not represented on the Amplicor strips. Sample #2764, as previously mentioned, contains HPV 59, an HPV genotype which is represented on the Amplicor strips. Therefore, this sample should be strip-positive for HPV 59. In addition, the probe sequences used in this assay were identical in sample #2764. There may be a few reasons for these discrepancies. First, the concentration of HPV DNA may be very low in these particular samples. Sensitivity may be an issue with this assay for HPV 59. Sensitivities vary between 10 and 100 genome copies per PCR reaction for types 6, 11, 16, 18, 31, 33, 45, 51, 52, 58, 59, 66 and 68 (Gravitt *et al.*, 1998). Nonetheless, Gravitt *et al.* (1998) have shown that discordant data for samples assessed by a dot blot assay and the Amplicor strip test were weakly dot blot positive, which suggests a low concentration of HPV DNA. Dot blots were not done on samples #2764 and #3069. However, a dot blot was done on #3111 and was positive at 65°C. The dot blots that were done in our lab were assessed only for positivity or negativity and were not designed to distinguish HPV type.

Coutlée *et al.* (1999) have also evaluated the line blot assay, in comparison to a dot blot assay, which serves as the gold standard for HPV typing. They found that this new assay was not as sensitive as the original consensus PCR which employed

radiolabelled oligonucleotides for typing, especially when samples are infected with more than one HPV genotype. In addition, they also demonstrated that the coamplification of HPV and β-globin reduced the level of sensitivity for HPV detection with consensus L1 primers. They suggested that this loss of sensitivity may in part be related to the batch synthesis of biotin-labelled primers. This synthesis was not controlled to ensure equivalent amounts of all degenerate primers. Therefore, inefficient amplification of some HPV genotypes may be a consequence of the underrepresentation of some degenerate primer pairs. Elimination of coamplification in reactions as well as modifying the amplification parameters by extending the amplification time resolved thirty-three out of thirty-seven samples that had been detected by standard dot blot but were undetected in a line blot assay. We have not attempted to examine these samples in a protocol which does not employ coamplification.

Multiple HPV Infected Samples

Amplicor data from samples #1378 and #1410 show multiple HPV infections by Amplicor strips. Unlike the other samples examined which were negative by Amplicor strips, samples #1378 and #1410 were sequenced to confirm the results found by ethidium bromide staining and Amplicor strip tests. Sample #1378 had three HPV types present; HPV 6, HPV 54 and HPV MM8. Sample #1410 had a double infection with HPV 16 and 51 (Table 2). Sequence data from these samples showed a single HPV type present; HPV 6 for #1378 and HPV 16 for #1410 (see Figures 8 and 10). Sample #1378

Numerous nucleotide changes were seen in the sequences from seven of the clones when compared to the prototype HPV CP6108 cloned L1 region. Twenty nucleotide changes are identical for all seven sequences in the 452bp L1 region of HPV CP6108 which makes these sequences more similar to each other than to the prototype. These changes occur at nucleotides 57, 75, 88, 111, 119, 120, 124, 128, 141, 147, 171, 174, 204, 229, 270, 281, 321, 347, 384 and 390 (Figure 11). Of these twenty nucleotide changes, nineteen amino acid changes are probable (ie. two of these changes are in the same reading frame). Specifically, three of these amino acid changes are non-conservative (THR→ALA, ASP→ASN, PRO→HIS) and the other sixteen amino acid changes are conservative. Figure 12 shows all of these amino acid changes in the 452bp region of HPV CP6108. Differences in amino acid sequence could lead to capsid protein differences and thus differences in folding.

These folding differences may be particularly significant in the three non-conservative amino acid changes. The first, occurring at nucleotide 124, threonine to alanine, causes the functional group of the amino acid to remain uncharged however, the change is from a larger hydrophilic (water-soluble) side chain to a smaller hydrophobic one. This may increase the flexibility of the protein at this point. The second non-conservative amino acid change, aspartic acid to asparagine, changes a smaller negatively charged functional group to a larger, uncharged, polar functional group. This change takes place at nucleotide 229 and may reduce the flexibility at this point in the L1 protein. The third amino acid change (proline → histidine) results in a gain of a positively charged

side chain. This will attract the negatively charged amino acid side chains in the area surrounding the asparagine at nucleotide 281 and therefore may alter the conformation of the protein. All of these amino acid changes could cause the tertiary folding of the L1 protein to change so that the regions responsible for receptor binding may be altered.

As an alternative explanation of the mixed sequence data for sample #121, it is also possible that more than one variant of HPV CP6108 is present in this individual. Several studies have shown the presence of more than one HPV 16 variant in an individual (Ho *et al.*, 1991, 1993b; Chan *et al.*, 1992; Xi *et al.*, 1993, 1995; Wheeler *et al.*, 1997). Chan *et al.* (1992) postulated that spontaneous mutations occur so rarely that a variant does not change during the lifespan of an infected person. If this hypothesis is true than the other variants present in this patient sample would most likely represent separate variants acquired during the infection rather than mutations of the initial variant.

To support the case of more than one variant present in an individual, Ho *et al.* (1991) suggest that the high frequency of single rather than duplicate point mutations in the sequenced region points to natural diversity rather than PCR errors. Therefore, it is likely that some of the clones from this sample are individual variants since the majority of nucleotide changes are point mutations. Mutations do occur during the PCR amplification process but are rare. It has been estimated that Taq polymerase has an error rate of 10^{-5} (Ho *et al.*, 1991; Xi *et al.*, 1995). However, Vent polymerase, which was used in our cloning experiments, has 3' to 5' exonuclease activity that is not present in Taq polymerase. Therefore, the mutational changes that occur are 3-15 times less frequent

and the mutation rate is lower than 10^{-5} (Ho *et al.*, 1991). Xi *et al.* (1995) showed that Vent polymerase introduced fewer mutations and the error rate is approximately 10^{-6}. To significantly reduce the possibility of obtaining variants created by PCR, several unique clones should be sequenced to find more than one of the same sequence and in addition, a reamplification should be done to confirm the sequences.

Rare HPV Genotypes

A phylogenetic tree of the papillomavirus family was originally created in 1995. In 1997, this tree contained 108 papillomavirus sequences based on the L1 consensus region from Manos primers. This tree is divided into five supergroups, A-E. Supergroups A and B are human papillomaviruses, with group A containing mucosal/genital HPV types and B cutaneous and Epidermodysplasia Verruciformis (EV) types. Group A3 is composed of HPV 61, 62, 72, MM7, MM8, A6053, CP6108 and CP8304 (Chan *et al.*, 1995; http://hpv-web.lanl.gov/). The majority of the samples sequenced in Table 2 were from group A3. HPV XS4, whose closest homology is HPV MM8, most likely is a member of this group too.

The prevalence of these genotypes is underestimated in studies and should be considered in HPV type-specific population studies. Most studies have included these rare HPV types within an unknown category termed HPV-X. Several studies have shown that the unknown HPV types represent a significant proportion of the total HPVs. In particular, Remmink *et al.* (1995) demonstrated that 15.0% of the total HPV positive

samples (n=227) were uncharacterized. These unknown HPV positives could be HPV 62, 70, 72, MM4, MM7, MM8, MM9, CP6108 or CP8304 (Appendix, Table 1). Burk *et al.* (1996) characterized thirty-nine HPV genotypes, twelve more than the Remmink group, and found that 21.0% of their HPV positive samples were unknown types in 157 HPV positive samples, however several samples had multiple HPV genotypes. The rare HPV types, HPV CP6108 and CP8304 were not tested for (Appendix, Table 2). Ho *et al.* (1998b) also looked at type-specific prevalence in HPV positive samples (n=262) and showed that 10.3% of their HPV positive samples were uncharacterized. This group looked for 37 HPV genotypes, including rare types, AE2, AE7 and AE8 (Appendix, Table 3). AE2 is a L1 sequence published by Tachezy *et al.* (1994) and was included in the analysis of these authors. AE7 and AE8 are novel HPV L1 sequences (unpublished). Probes have not been generally used to assess for HPV 62, CP6108 and CP8304 in study populations.

Meyer *et al.* (1998) conducted a study to obtain more information about the prevalence and association of rare HPV types (HPV 59, 61, 62, 66, 70, CP6108, CP8304, MM4, MM7, MM8 and MM9). They found that taken individually, these HPV genotypes occur very rarely. However, when considered collectively, these rare genotypes contribute a significant proportion (~10%) of all HPV infections in premalignant lesions. New variations of the line blot assay will include probes for other genotypes.

Currently, HPV DNA typing is not used in patient management and cancer

screening. However, in the near future, this may be modified. The success of this testing will depend on the detection assays used. The Amplicor assay is compatible with large population studies and screening. To effectively and rapidly discriminate between high and low risk HPV types and more specifically amongst twenty-seven or more HPV genotypes will support the use of this assay to influence patient treatment in the future.

CHAPTER 2: THE PERSISTENCE OF HUMAN PAPILLOMAVIRUS

INTRODUCTION

The results described here relate to a subset of patients who had evidence of HPV of the same genotype on subsequent occasions over a three year study period. These studies form the theoretical basis for a discussion of the most appropriate definition of HPV persistence and eventually, the association of persistence with clinical pathology.

In particular, we have examined a number of patients whose samples were found to be HPV 16 positive on a number of visits. In addition, we investigated patients who had evidence of HPV 18 on subsequent visits, patients who were positive for HPV 6 infection on subsequent occasions and one patient positive for HPV 66 on her initial and follow-up visit.

RESULTS

Fifteen samples acquired from six students attending the University of Toronto health clinic for reasons other than gynecological concerns were assessed for HPV infection by three methods. The methods were described previously in the results section of Chapter 1. The summary of data obtained from the six patients is presented in Table 5.

For each of the HPV 16 positive samples in Table 5, the PCR product of the viral LCR was sequenced (Franco *et al.*, 1994). The sequences obtained from each of the samples of individual patients were aligned to each other and are shown in Figures 14 and 15. First annual samples, numbers 3155 and 3174 could not be sequenced. Table 6 lists the same six patients and identifies the samples for which an LCR region sequence was obtained.

In order to determine how many HPV 16 LCR alleles were in the six patients, the LCR region sequences were compared. If follow up samples from a patient were identical to the baseline samples in LCR sequence, only one of the two samples was compared to other patient samples. Dots above the sequence represent a nucleotide change in the sequence. The LCR sequence comparisons in the six patients are shown in Figures 16 to 23. Each of the LCR sequences were compared to the HPV 16 prototype sequence (Seedorf *et al.*, 1985). The sequence comparisons are illustrated in Figures 24 and 25. The summary of HPV 16 LCR alleles found in the ten sequences is presented in Table 7; two alleles were observed.

Thirteen samples were obtained from five other patients who had evidence of HPV types other than HPV 16. The summary of HPV typing data obtained for each sample is shown in Table 8. For each of the patient samples that were positive for HPV of the same genotype on subsequent occasions, the PCR product was sequenced in the L1 region using Manos primers (Manos *et al.*, 1989). The sequences acquired from each of the samples in the same patient were aligned to each other and shown in Figures 26, 27

and 28. The HPV type is as stated in the figures. Sample #F114 is not shown in these figures because baseline sample #568 could not be sequenced. First annual sample #2516 was also unable to be sequenced.

Table 9 summarizes the data interpretation from the five patients for which the L1 region was sequenced. To determine the number of L1 alleles in the patients, each of the L1 sequences were compared to their respective prototypes (unpublished, http://hpv-web.lanl.gov/; Cole *et al.*, 1987; Schwarz *et al.*, 1983). If samples from the same patient were identical in L1 sequence, only one of the two or three sequences was subsequently compared to the prototype. Dots above the sequence represent a nucleotide change in the sequence. These sequence comparisons are shown in Figures 29, 30 and 31. The summary of the L1 alleles for each different genotype found in the patient samples is presented in Table 10. One allele was observed for HPV 66 and two alleles for both HPV 6 and 18.

The L1 sequences were compared for the two patients who were HPV 18 positive (Figure 32) and the two patients who were HPV 6 positive (Figure 33).

LITERATURE PERSPECTIVE (APPENDIX)

Twelve studies, from 1991 to present, were analysed for HPV type-specific prevalence. The patients in these studies were from different parts of the world and different populations, including patients attending university health clinics and patients who have or had cervical cancer. The published results, with respect to HPV type-

specific prevalence, are summarized in Tables 1, 2 and 3 of the Appendix. Also included in Table 1 is the HPV data acquired in our laboratory. The studies examined HPV prevalence for up to 46 HPV genotypes.

In order to determine the frequency of specific HPV alleles from several genotypes, particularly those types found in our study, a detailed compilation of relevant literature was undertaken. This included both published and unpublished data (http://htp-web.lanl.gov/). The information has been compiled in tables in the Appendix. Numerous variants and subtypes of the genotypes are evident and where the variants and subtypes have been compared to the original prototype, the homology is as indicated. We have also included, in the tables, the nucleotide position(s) that is/are changed from the prototype, the specific genes that the sequence is derived from and the number of strains that are found in the particular study. The frequency of an allele is defined as the number of specific alleles (variants) derived from a particular region within the HPV gene (LCR for HPV 16; L1 for HPV 18, 6 and 66) in relation to the total number of HPV strains found in the same region.

HPV 16 is the genotype that has been most extensively studied. Several studies have examined variants and subtypes from most of the genes of this genome. The data compiled for HPV 16 is in Table 4. A difficulty in comparing the variants and subtypes of HPV 16, particularly in the E2, E5, L1, L2 and LCR genes arises in the alignment of the sequences with the prototype (Seedorf *et al.*, 1985). Adjustments in nucleotide numbering are as described in the table.

For the purpose of this study, we examined a 404bp fragment in the LCR region. The frequency of HPV 16 alleles in this region was estimated from the compiled studies, representing 419 strains (Table 5).

The variants and subtypes of HPV 18 were also compiled (Table 6). Fewer studies have been completed on HPV 18 with respect to sequence analysis of variants and subtypes, although HPV 18 is found in a significant proportion of cervical cancers (Lowy *et al.*, 1994; Howley *et al.*, 1996; Bonnez, 1997). We examined the L1 region (450bp fragment) for HPV 18 and the frequency was estimated in 68 strains (Table 7). The L1 region has only been sequenced by two groups (Stewart *et al.*, 1996; Lizano *et al.*, 1997).

Variants and subtypes of HPV 6, the most common 'low risk' HPV type, are presented in Table 8. Fewer studies are available for compilation. The L1 region for HPV 6 has been analysed for 35 strains in two studies (Icenogle *et al.*, 1991; Caparrós-Wanderley *et al.*, 1999). A summary of the frequencies is presented in Table 9.

HPV 66 is a rare genotype which includes only two variants and has not been extensively examined (Table 10). The number of strains for one of the two variants is unknown and therefore, the frequency of HPV 66 L1 strains could not be estimated with the limited data.

Two more tables of variants and subtypes were compiled for HPV 57 (Table 11) and HPV 58 (Table 12). Only one study was published for each of these genotypes (Chan *et al.*, 1997; Stewart *et al.*, 1996). Although we did not specifically examine HPV 57 and 58 in our study, we wanted to look at the availability of sequence information of

two other HPV types. These genotypes varied greatly in type-specific prevalence (Tables 1, 2 and 3).

TABLE 5: RESULTS FOR SAMPLES WHICH WERE INFECTED WITH HPV 16

Baseline #	Ethidium Bromide	Dot Blot 55°	Dot Blot 65°	Amplicor Results	First Annual #	Ethidium Bromide	Amplicor Results	Second Annual #	Ethidium Bromide	Amplicor Results
263	M+,16+	-	-	16+	1659	16+	16+	2789	-ve	-ve
621	G+, M+, 16+	+	+	16+	1962	M+,16+	16+	2994	-ve	-ve
697	M+	+	+	16+	1985	-ve	16+	2833	-ve	-ve
849	M+, 16+	+	+	16+	2226	M+, 16+	16+	----	----	----
1351	16+	-	-	16+, 58+	3155	M+	16+,45+, 58+, MM8+	----	----	----
2141	M+, 16+	+	+	16+, 56+	3174	-ve	16+	----	----	----

M+ = positive by Manos primers (L1 consensus primers)
G+ = positive by Gregoire primers (E1 consensus primers)
N.D. = not done
D.B. = dot blot ; dot blot has 5 probe mixture of HPV 6, 11, 16, 18, 33

FIGURE 14: HPV 16 LCR REGION SEQUENCES
A COMPARISON FOR SAME PATIENT SAMPLES ON SUBSEQUENT OCCASIONS
(BASELINE SAMPLES #263 AND #621)

1659 TGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACCTGCACTGCTTGCCAACCATT
263 TGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACCTGCACTGCTTGCCAACCATT

CCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATCACTATGCGCCAACGCCTTA
CCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATCACTATGCGCCAACGCCTTA

CATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAACTTCTAAGGCCAACTAAA
CATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAACTTCTAAGGCCAACTAAA

TGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGGTGTGTGCAAACCG
TGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGGTGTGTGCAAACCG

HOMOLOGY = 100%

1962 GTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACCTGCACTGCTTGCCA
621 GTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACCTGCACTGCTTGCCA

ACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATCACTATGCGCCAAC
ACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATCACTATGCGCCAAC

GCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAACTTCTAAGGCCAA
GCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAACTTCTAAGGCCAA

CTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGGTGTGTGCAAACCG
CTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGGTGTGTGCAAACCG

HOMOLOGY = 100%

Underlined sequence is primer

FIGURE 15: HPV 16 LCR REGION SEQUENCES
A COMPARISON FOR SAME PATIENT SAMPLES ON SUBSEQUENT OCCASIONS
(BASELINE SAMPLES #697 AND #849)

1985 TTTAAATAGTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACCTGCACT
697 TTTAAATAGTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACCTGCACT

GCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATCACTAT
GCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATCACTAT

GCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAACTTC
GCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAACTTC

TAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGGTGT
TAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGGTGT

GTGCAAACCG
GTGCAAACCG

HOMOLOGY = 100%

2226 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGAC
849 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGAC

TGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATC
TGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATC

ACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAA
ACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAA

CTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGG
CTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGG

TGTGTGCAAAACCG
TGTGTGCAAAACCG

HOMOLOGY = 100%

Underlined sequence is primer

TABLE 6: AMPLICOR AND SEQUENCE RESULTS (LCR REGION) FOR SAMPLES WHICH WERE INFECTED WITH HPV 16

Baseline #	Amplicor Results	First Annual #	Amplicor Results	Second Annual #	Amplicor Results	Identical Sequences in the LCR Region
263 ✯	16+	1659 ✯	16+	2789	-ve	Yes
621 ✯	16+	1962 ✯	16+	2994	-ve	Yes
697 ✯	16+	1985 ✯	16+	2833	-ve	Yes
849 ✯	16+	2226 ✯	16+	----	----	Yes
1351 ✯	16+, 58+	3155	16+,45+, 58+, MM8+	----	----	----
2141 ✯	16+, 56+	3174	16+	----	----	----

✯ LCR region sequenced

FIGURE 16: HPV 16 LCR REGION SEQUENCES
A COMPARISON BETWEEN PATIENT SAMPLES (#621/#1659 AND #621/#697)

1659 GTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACCTGCACTGCTTGCCA
621 GTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACCTGCACTGCTTGCCA

ACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATCACTATGCGCCAAC
ACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATCACTATGCGCCAAC

GCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAACTTCTAAGGCCAA
GCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAACTTCTAAGGCCAA

CTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGGTGTGTGCAAACCG
CTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGGTGTGTGCAAACCG

HOMOLOGY = 100%

697 GTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACCTGCACTGCTTGCCA
621 GTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACCTGCACTGCTTGCCA

ACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATCACTATGCGCCAAC
ACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATCACTATGCGCCAAC

GCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAACTTCTAAGGCCAA
GCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAACTTCTAAGGCCAA

CTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGGTGTGTGCAAACCG
CTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGGTGTGTGCAAACCG

HOMOLOGY = 100%

Underlined sequence is primer

FIGURE 17: HPV 16 LCR REGION SEQUENCES
A COMPARISON BETWEEN PATIENT SAMPLES (#697/#1659 AND #621/#849)

697 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACC
1659 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACC

TGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATC
TGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATC

ACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAA
ACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAA

CTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGG
CTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGG

TGTGTGCAAACCG
TGTGTGCAAACCG

HOMOLOGY = 100%

849 GTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACCTGCACTGCTTGCCA
621 GTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACCTGCACTGCTTGCCA

ACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATCACTATGCGCCAAC
ACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATCACTATGCGCCAAC

GCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAACTTCTAAGGCCAA
GCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAACTTCTAAGGCCAA

CTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGGTGTGTGCAAAACCG
CTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGGTGTGTGCAAA -CCG

HOMOLOGY = 100%

Underlined sequence is primer

FIGURE 18: HPV 16 LCR REGION SEQUENCES
A COMPARISON BETWEEN PATIENT SAMPLES (#621/#2141 AND #621/#1351)

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2141 GTTCTATGTCAGCAACTATGGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACCTGCACTGCTTGCCA
621 GTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACCTGCACTGCTTGCCA

ACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATCACTATGCGCCAAC
ACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATCACTATGCGCCAAC

GCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAACTTCTAAGGCCA
GCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAACTTCTAAGGCCA

ACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGGTGTGTGCAAAA
ACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGGTGTGTGCAAA -

CCG
CCG

HOMOLOGY = 99.7%

•

1351 GTTCTATGTCAGCAACTATGGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACCTGCACTGCTTGCCA
621 GTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACCTGCACTGCTTGCCA

ACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATCACTATGCGCCAAC
ACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATCACTATGCGCCAAC

GCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAACTTCTAAGGCCAA
GCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAACTTCTAAGGCCAA

CTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGGTGTGTGCAAACCG
CTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGGTGTGTGCAAACCG

HOMOLOGY = 99.7%

• represents a nucleotide change between sequences Underlined sequence is primer

FIGURE 19: HPV 16 LCR REGION SEQUENCES
A COMPARISON BETWEEN PATIENT SAMPLES (#849/#1659 AND #1351/#1659)

849 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGAC
1659 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGAC

CTGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAAT
CTGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAAT

CACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAA
CACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAA

ACTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGG
ACTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGG

GTGTGTGCAAAACCG
GTGTGTGCAAA - CCG

HOMOLOGY = 100%

•

1351 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATGGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGAC
1659 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGAC

CTGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAAT
CTGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAAT

CACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAA
CACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAA

ACTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGG
ACTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGG

GTGTGTGCAAACCG
GTGTGTGCAAACCG

HOMOLOGY = 99.7%

• represents a nucleotide change between sequences

Underlined sequence is primer

FIGURE 20: HPV 16 LCR REGION SEQUENCES
A COMPARISON BETWEEN PATIENT SAMPLES (#1659/#2141 AND #697/#849)

•

2141 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATGGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACC
1659 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACC

TGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATC
TGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATC

ACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAA
ACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAA

CTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGG
CTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGG

TGTGTGCAAAACCG
TGTGTGCAAA - CCG

HOMOLOGY = 99.7%

849 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGAC
697 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGAC

CTGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAA
CTGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAA

TCACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTT
TCACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTT

AAACTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACA
AAACTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACA

TGGGTGTGTGCAAAACCG
TGGGTGTGTGCAAA - CCG

HOMOLOGY = 100%

• represents a nucleotide change between sequences Underlined sequence is primer

FIGURE 21: HPV 16 LCR REGION SEQUENCES
A COMPARISON BETWEEN PATIENT SAMPLES (#697/#1351 AND #697/#2141)

•

1351 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATGGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGA
697 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGA

CCTGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAA
CCTGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAA

ATCACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTT
ATCACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTT

TAAACTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCAC
TAAACTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCAC

ATGGGTGTGTGCAAACCG
ATGGGTGTGTGCAAACCG

HOMOLOGY = 99.7%

•

2141 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATGGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGAC
697 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGAC

CTGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAA
CTGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAA

TCACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTT
TCACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTT

AAACTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACA
AAACTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACA

TGGGTGTGTGCAAAACCG
TGGGTGTGTGCAAA - CCG

HOMOLOGY = 99.7%

• represents a nucleotide change between sequences

Underlined sequence is primer

FIGURE 22: HPV 16 LCR REGION SEQUENCES
A COMPARISON BETWEEN PATIENT SAMPLES (#849/#1351 AND #849/#2141)

•

1351 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATGGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGAC
849 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGAC

CTGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAAT
CTGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAAT

CACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAA
CACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAA

ACTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGG
ACTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGG

GTGTGTGCAAA- CCG
GTGTGTGCAAAACCG

HOMOLOGY = 99.7%

•

2141 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATGGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGAC
849 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGAC

CTGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAA
CTGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAA

TCACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTT
TCACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTT

AAACTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACA
AAACTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACA

TGGGTGTGTGCAAAACCG
TGGGTGTGTGCAAAACCG

HOMOLOGY = 99.7%

• represents a nucleotide change between sequences Underlined sequence is primer

FIGURE 23: HPV 16 LCR REGION SEQUENCES
A COMPARISON BETWEEN PATIENT SAMPLES (#1351/#2141)

2141 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATGGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGAC
1351 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATGGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGAC

CTGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAAT
CTGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAAT

CACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAA
CACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAA

ACTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGG
ACTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGG

GTGTGTGCAAAACCG
GTGTGTGCAAA - CCG

HOMOLOGY = 100%

Underlined sequence is primer

FIGURE 24: LCR SEQUENCE DATA
A COMPARISON TO THE HPV 16 PROTOTYPE (SAMPLES #621, #697, #849 AND #1659)

•

621 GTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACCTGCACTGCTTGCCA
HPV 16 [7499]GTTCTATGTCAGCAACTATGGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACCTGCACTGCTTGCCA

ACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATCACTATGCGCCAAC
ACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATCACTATGCGCCAAC

GCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAACTTCTAAGGCCAA
GCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAACTTCTAAGGCCAA

CTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGGTGTGTGCAAACCG
CTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGGTGTGTGCAAACCG[7660]

HOMOLOGY = 99.7%

697

849 •

1659 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATAGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACC
HPV 16 [7484]GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATGGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGACC

TGACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATCA
TGACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAAATCA

CTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAAC
CTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTTTAAAC

TTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGGT
TTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGGT

GTGTGCAAACCG
GTGTGCAAACCG[7660]

HOMOLOGY = 99.7%

• represents a nucleotide change between sequences

Underlined sequence is primer

FIGURE 25: LCR SEQUENCE DATA
A COMPARISON TO THE HPV 16 PROTOTYPE (SAMPLES #1351 AND #2141)

1351
2141 GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATGGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGA
HPV 16 7484GTGTTTTTTTAAATAGTTCTATGTCAGCAACTATGGTTTAAACTTGTACGTTTCCTGCTTGCCATGCGTGCCAAATCCCTGTTTTCCTGA

CCTGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAA
CCTGCACTGCTTGCCAACCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGTGTCATATAAAATAA

ATCACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTT
ATCACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCTTGTTTTAACTAACCTAATTGCATATTTGGCATAAGGTT

TAAACTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCAC
TAAACTTCTAAGGCCAACTAAATGTCACCCTAGTTCATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCAC

ATGGGTGTGTGCAAAACCG
ATGGGTGTGTGCAAA - CCG7860

HOMOLOGY = 100%

Underlined sequence is primer

TABLE 7: LONG CONTROL REGION (LCR) ALLELES FOR HPV 16

HPV 16 LCR ALLELE	SAMPLE #
HPV 16 prototype	1351 2141
A_{7518}	263 621 697 849 1659 1962 1985 2226

Note: 7518 refers to the nucleotide position in the long control region of HPV 16 that is changed from the original prototype sequence (Seedorf *et al.*, 1985).

TABLE 8: RESULTS FOR SAMPLES WHICH WERE INFECTED WITH HPV TYPES OTHER THAN HPV 16

Baseline #	Ethidium Bromide	Dot Blot 55°	Dot Blot 65°	Amplicor Results	First Annual #	Ethidium Bromide	Amplicor Results	Second Annual #	Ethidium Bromide	Amplicor Results
973	16+	-	-	66+	2920	M+, 16+	66+	----	----	----
1146	M+	+	+	18+, 53+	2516	M+	18+, 31+, 53+	3299	M+	18+
210	6/11+	-	+	6+, 54+, MM8+	1957	M+	18+	3042	M+, 16+	18+
568	M+, 6/11+, 16+	+	+	6+, 33+, 52+, 58+	F114	M+	6+, 52+, 58+	----	----	----
1175	6/11+	+	+	6+	2392	M+, 6/11+	6+, 54+	3157	M+	6+

M+ = positive by Manos primers (L1 consensus primers)
G+ = positive by Gregoire primers (E1 consensus primers)
N.D. = not done
D.B. = dot blot ; dot blot has 5 probe mixture of HPV 6, 11, 16, 18, 33

FIGURE 26: L1 REGION SEQUENCE DATA (FOR HPV TYPES OTHER THAN HPV 16) A COMPARISON FOR SAME PATIENT SAMPLES ON SUBSEQUENT OCCASIONS (BASELINE SAMPLES #973 AND #1146)

973 CCTGTAAATTAACCTCCCAAAACTTATATTTAGCCAGGGGATCCTGCTTTTCTGCAGGGGGCTGTTCCCTTTGACATGTAATAGCTGTGCT
2920 CCTGTAAATTAACCTCCCAAAACTTATATTTAGCCAGGGGATCCTGCTTTTCTGCAGGGGGCTGTTCCCTTTGACATGTAATAGCTGTGCT

TTTAATATACCTATATTTATCCTCTAAGCTAGTTGCAACTGGTGGGGACAATCCAATGTTCCAATCGTCTAATAAAGTATTATTCATATTA
TTTAATATACCTATATTTATCCTCTAAGCTAGTTGCAACTGGTGGGGACAATCCAATGTTCCAATCGTCTAATAAAGTATTATTCATATTA

TGCAAATATGCCATAACTTCTGCAGTTAAGGTTATTTTACAAAGTTGAAACACAAACTGTAGTTCATATTCCTCCACATGGCGAAGGTAT
TGCAAATATGCCATAACTTCTGCAGTTAAGGTTATTTTACAAAGTTGAAACACAAACTGTAGTTCATATTCCTCCACATGGCGAAGGTAT

TGATTGATTTCACGTGCATCATATTTAGTTAATGTGCTTTTAGCTGCATTAATAGTCATGTTGGTACTTCTGGTAGTATCCACAACAGTAA
TGATTGATTTCACGTGCATCATATTTAGTTAATGTGCTTTTAGCTGCATTAATAGTCATGTTGGTACTTCTGGTAGTATCCACAACAGTAA

CAAATACCTGATTACCCCAGCATATGCCATTATTATGACCCTTGGGC
CAAATACCTGATTACCCCAGCATATGCCATTATTATGACC-TTGGGC

TYPE: HPV 66 VARIANT **HOMOLOGY = 100%**

1146 AAAACTTTTCCTTTAAATCCACATTCCAAAACTTTAACTTATCATAGGGATCCTTATTTTCAGCCGGTGCAGCATCCTTTTGACAGGTAAT
3299 AAAACTTTTCCTTTAAATCCACATTCCAAAACTTTAACTTATCATAGGGATCCTTATTTTCAGCCGGTGCAGCATCCTTTTGACAGGTAAT

AGCAACAGATTGTACAAAACGATATGTATCCACCAAACTAGTAGTTGGCGGGGGGGGGAACACCAAAGTTCCAATCCTCTAAAATACTGC
AGCAACAGATTGTACAAAACGATATGTATCCACCAAACTAGTAGTTGGCGGGGGGGGGAACACCAAAGTTCCAATCCTCTAAAATACTGC

TATTCATACTATGAATATAGGACATAACATCTGCAGTTAAAGTAATAGTACACAACTGAAAAATAAACTGCAAATCATATTCCTCAACAT
TATTCATACTATGAATATAGGACATAACATCTGCAGTTAAAGTAATAGTACACAACTGAAAAATAAACTGCAAATCATATTCCTCAACAT

GTCTGCTATACTGCTTAAATTTGGTAGCATCATATTGCCCAGGTACAGGAGACTGTGTAGAAGCACATATTGTTAAATTGGTACTGCGAG
GTCTGCTATACTGCTTAAATTTGGTAGCATCATATTGCCCAGGTACAGGAGACTGTGTAGAAGCACATATTGTTAAATTGGTACTGCGAG

TGGTATCTACCACAGTAACAAATAATTGATTATGCCAGCAAACACCATTGTTATGACCCTGG -GC
TGGTATCTACCACAGTAACAAATAATTGATTATGCCAGCAAACACCATTGTTATGACCCTTGGGC
TYPE: HPV 18 VARIANT **HOMOLOGY = 100%** Underlined sequence is primer

FIGURE 27: L1 REGION SEQUENCE DATA (FOR HPV TYPES OTHER THAN HPV 16) A COMPARISON FOR SAME PATIENT SAMPLES ON SUBSEQUENT OCCASIONS (BASELINE SAMPLE #1957)

1957 TTCCTTTAAATCCACATTCCAAAACTTTAACTTATCATAGGGATCCTTATTTTCAGCTGGTGCAGCATCCTTTTGACAGGTAATAGCAACA
3042 TTCCTTTAAATCCACATTCCAAAACTTTAACTTATCATAGGGATCCTTATTTTCAGCTGGTGCAGCATCCTTTTGACAGGTAATAGCAACA

GATTGTACAAAACGATATGTATCCACCAAACTAGTAGTTGGCGGGGGGGGGAACACCAAAGTTCCAATCCTCTAAAATACTGCTATTCAT
GATTGTACAAAACGATATGTATCCACCAAACTAGTAGTTGGCGGGGGGGGGAACACCAAAGTTCCAATCCTCTAAAATACTGCTATTCAT

ACTATGAATATAGGACATAACATCTGCAGTTAAAGTAATAGTACATAACTGAAAAATAAACTGCAAATCATATTCTTCAACATGTCTGC
ACTATGAATATAGGACATAACATCTGCAGTTAAAGTAATAGTACATAACTGAAAAATAAACTGCAAATCATATTCTTCAACATGTCTGC

TATACTGCTTAAATTTGGTAGCATCATATTGCCCAGGTACAGGAGACTGTGTAGAAGCACATATTGTTAAATTGGTACTACGAGTGGTAT
TATACTGCTTAAATTTGGTAGCATCATATTGCCCAGGTACAGGAGACTGTGTAGAAGCACATATTGTTAAATTGGTACTACGAGTGGTAT

CTACCACAGTAACAAATAATTGATTATGCCAGCAGATACCATTGTTATGACCCTTGGGC
CTACCACAGTAACAAATAATTGATTATGCCAGCAGATACCATTGTTATGACCCCGGGGC

TYPE: HPV 18 VARIANT

HOMOLOGY = 100%

Underlined sequence is primer

FIGURE 28: L1 REGION SEQUENCE DATA (FOR HPV TYPES OTHER THAN HPV 16) A COMPARISON FOR SAME PATIENT SAMPLES ON SUBSEQUENT OCCASIONS (BASELINE SAMPLE #1175)

1175 TAGAAAACTTTTCTTTTAAATTAACCTCCCAAAAACTAAGGTTCTTATAGGGATCTGGCTTTTCCTTTTCAGGAGTGGGCTTTTGACAGGT
2392 TAGAAAACTTTTCTTTTAAATTAACCTCCCAAAAACTAAGGTTCTTATAGGGATCTGGCTTTTCCTTTTCAGGAGTGGGCTTTTGACAGGT
3157 TAGAAAACTTTTCTTTTAAATTAACCTCCCAAAAACTAAGGTTCTTATAGGGATCTGGCTTTTCCTTTTCAGGAGTGGGCTTTTGACAGGT

AATGGCCTGTGACTGCACATACCTATAGGTATCTTCTAATGTACCATTTGGGGGAGGCGATAACCCAAAGTTCCAGTCTTCCAAAACAGA
AATGGCCTGTGACTGCACATACCTATAGGTATCTTCTAATGTACCATTTGGGGGAGGCGATAACCCAAAGTTCCAGTCTTCCAAAACAGA
AATGGCCTGTGACTGCACATACCTATAGGTATCTTCTAATGTACCATTTGGGGGAGGCGATAACCCAAAGTTCCAGTCTTCCAAAACAGA

GGGATTCATTGTGTGAATATAGGCCATTACTTCAGCAGACAATGTAATGCTACATAATTGAAAAATAAATTGTAAATCATACTCTTCCAC
GGGATTCATTGTGTGAATATAGGCCATTACTTCAGCAGACAATGTAATGCTACATAATTGAAAAATAAATTGTAAATCATACTCTTCCAC
GGGATTCATTGTGTGAATATAGGCCATTACTTCAGCAGACAATGTAATGCTACATAATTGAAAAATAAATTGTAAATCATACTCTTCCAC

ATGACGCATGTACTCTTTATAATCAGAATTGGTGTATGTGGAAGATGTAGTTACGGATGCACATAATGTCATGTTGGTACTGCGTGTGGT
ATGACGCATGTACTCTTTATAATCAGAATTGGTGTATGTGGAAGATGTAGTTACGGATGCACATAATGTCATGTTGGTACTGCGTGTGGT
ATGACGCATGTACTCTTTATAATCAGAATTGGTGTATGTGGAAGATGTAGTTACGGATGCACATAATGTCATGTTGGTACTGCGTGTGGT

ATCTACCACAGTAACAAACAGTTGATTACCCCAACAAATACCATTGTTATGACCCTGGGC
ATCTACCACAGTAACAAACAGTTGATTACCCCAACAAATACCATTGTTATGACCCTGGGC
ATCTACCACAGTAACAAACAGTTGATTACCCCAACAAATACCATTGTTATGACCCTGGGC

TYPE: HPV 6b

HOMOLOGY = 100%

Underlined sequence is primer

TABLE 9: AMPLICOR AND SEQUENCE RESULTS (L1 REGION) FOR SAMPLES WHICH WERE INFECTED WITH HPV TYPES OTHER THAN HPV 16

Baseline #	Amplicor Results	First Annual #	Amplicor Results	Second Annual #	Amplicor Results	Identical Sequences in the L1 Region
973 ✯	66+	2920 ✯	66+	----	----	Yes
1146 ✯	18+, 53+	2516	18+, 31+, 53+	3299 ✯	18+	Yes
210	6+, 54+, MM8+	1957 ✯	18+	3042 ✯	18+	Yes
568	6+, 33+, 52+, 58+	F114 ✯	6+, 52+, 58+	----	----	----
1175 ✯	6+	2392 ✯	6+, 54+	3157 ✯	6+	Yes

✯ L1 region sequenced

FIGURE 29: A COMPARISON OF THE L1 REGION TO PROTOTYPES (FOR HPV TYPES OTHER THAN HPV 16) (BASELINE SAMPLES #973 AND #1146)

973 CCTGTAAATTAACCTCCCAAAACTTATATTTAGCCAGGGGATCCTGCTTTTCTGCAGGGGGCTGTTCCCTTTGACATGTAATAGCTGTGC
HPV 66 6997CCTGTAAATTAACTTCCCAAAACTTATATTTAGCCAGGGGATCCTGCTTTTCTGCAGGGGGCTGTTCCCTCTGACATGTAATAGCTGTGC

TTTTAATATACCTATATTTATCCTCTAAGCTAGTTGCAACTGGTGGGGACAATCCAATGTTCCAATCGTCTAATAAAGTATTATTCATATT
TTTTAATATACCTATATTTATCCTCTAAGCTAGTTGCAACTGGTGGGGATAAGCCAATATTCCAATCGTCTAATAAAGTATTATTCATATT

ATGCAAATATGCCATAACTTCTGCAGTTAAGGTTATTTTACAAAGTTGAAACACAAACTGTAGTTCATATTCCTCCACATGGCGAAGGTA
ATGCAAATATGCCATAACTTCTGCAGTTAAGGTTATTTTACAAAGTTGAAACACAAACTGTAGTTCATATTCCTCCACATGGCGAAGGTA

TTGATTGATTTCACGTGCATCATATTTAGTTAATGTGCTTTTAGCTGCATTAATAGTCATGTTGGTACTTCTGGTAGTATCCACAACAGTA
TTGATTGATTTCACGGGCATCATATTTAGTTAATGTGCTTTTAGCTGCATTAATAGTCATGTTGGTGCTTCTGGTAGTATCCACAACAGTA

ACAAATACCTGATTACCCCAGCATATGCCATTATTATGACCTTGGGC
ACAAATACCTGATTACCCCAGCATATGCCATTATTATGGCCCTGTGC6589

HOMOLOGY = 98.2%

1146 AAAACTTTTCCTTTAAATCCACATTCCAAAACTTTAACTTATCATAGGGATCCTTATTTTCAGCCGGTGCAGCATCCTTTTGACAGGTAA
HPV 18 6981AAAACTTTTCCTTTAAATCCACATTCCAAAACTTTAACTTATCATAGGGATCCTTATTTTCAGCCGGTGCAGCATCCTTTTGACAGGTAA

TAGCAACAGATTGTACAAAACGATATGTATCCACCAAACTAGTAGTTGGCGGGGGGGGAACACCAAAGTTCCAATCCTCTAAAATACTG
TAGCAACAGATTGTACAAAACGATATGTATCCACCAAACTAGTAGTTGGGGGGGGGGGAACACCAAAGTTCCAATCCTCTAAAATACTG

CTATTCATACTATGAATATAGGACATAACATCTGCAGTTAAAGTAATAGTACACAACTGAAAAATAAACTGCAAATCATATTCCTCAACA
CTATTCATACTATGAATATAGGACATAACATCTGCAGTTAAAGTAATAGTACACAACTGAAAAATAAACTGCAAATCATATTCCTCAACA

TGTCTGCTATACTGCTTAAATTTGGTAGCATCATATTGCCCAGGTACAGGAGACTGTGTAGAAGCACATATTGTTAAATTGGTACTGCGA
TGTCTGCTATACTGCTTAAATTTGGTAGCATCATATTGCCCAGGTACAGGAGACTGTGTAGAAGCACATATTGTTAAATTGGTACTGGGA

GTGGTATCTACCACAGTAACAAATAATTGATTATGCCAGCAAACACCATTGTTATGACCCTTGGC
GTGGTATCTACCACAGTAACAAATAATTGATTATGCCAGCAAACACCATTGTTATGACCCTGTGC4558

HOMOLOGY = 99.5%

• represents a nucleotide change between sequences

Underlined sequence is primer

FIGURE 30: A COMPARISON OF THE L1 REGION TO PROTOTYPES (FOR HPV TYPES OTHER THAN HPV 16) (BASELINE SAMPLES #1175 AND #1957)

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FIGURE 31: A COMPARISON OF THE L1 REGION TO PROTOTYPES (FOR HPV TYPES OTHER THAN HPV 16) (SAMPLE #F114)

F114 TAGAAAACTTTTCTTTTAAATTAACCTCCCAAAAACTAAGGTTCTTATAGGGATCTGGCTTTTGCTTTTCAGGAGTGGGCTTTTGACAGG

HPV 6b 7142TAGAAAACTTTTCTTTTAAATTAACCTCCCAAAAACTAAGGTTCTTATAGGGATCTGGCTTTTCCTTTTCAGGAGTGGGCTTTTGACAGG

TAATGGCCTGTGACTGCACATACCTATAGGTATCTTCTAATGTACCATTTGGGGGAGGCGATAACCCAAAGTTCCAGTCTTCCAAAACA
TAATGGCCTGTGACTGCACATACCTATAGGTATCTTCTAATGTACCATTTGGGGGAGGCGATAACCCAAAGTTCCAGTCTTCCAAAACA

GAGGGATTCATTGTGTGAATATAGGCCATTACTTCAGCAGACAATGTAATGCTACATAATTGAAAAATAAATTGTAAATCATACTCTTC
GAGGGATTCATTGTGTGAATATAGGCCATTACTTCAGCAGACAATGTAATGCTACATAATTGAAAAATAAATTGTAAATCATACTCTTC

CACATGACGCATGTACTCTTTATAATCAGAATTGGTGTATGTGGAAGATGTAGTTACGGATGCACATAATGTCATGTTGGTACTGCGTG
CACATGACGCATGTACTCTTTATAATCAGAATTGGTGTATGTGGAAGATGTAGTTACGGATGCACATAATGTCATGTTGGTACTGCGTG

TGGTATCTACCACAGTAACAAACAGTTGATTACCCCAACAAATACCATTGTTATGTCCCTGGGC
TGGTATCTACCACAGTAACAAACAGTTGATTACCCCAACAAATACCATTGTTATGTCCCTGGGC6722

HOMOLOGY = 99.8%

• represents a nucleotide change between sequences
Underlined sequence is primer

TABLE 10: L1 ALLELES FOR HPV TYPES OTHER THAN HPV 16

HPV GENOTYPE	L1 ALLELE	SAMPLE #
HPV 66	$A_{6660, 6711, 6849, 6855, 6858, 6927, 6984}$	973 2920
HPV 18	$A_{6625, 6842}$	1146 3299
HPV 18	$A_{6579, 6581, 6625, 6626, 6719, 6749, 6842, 6917}$	1957 3042
HPV 6b	prototype	1175 2392 3157
HPV 6b	A_{7079}	F114

Note: For HPV 66; 6660, 6711, 6849, 6855, 6858, 6927 and 6984 refer to the nucleotide positions in the L1 region that are changed from the prototype sequence (unpublished, http://htp-web.lanl.gov/).
For HPV 18; 6579, 6581, 6625, 6626, 6719, 6749, 6842 and 6917 refer to the nucleotide positions in the L1 region that are changed from the prototype sequence (Cole *et al.*, 1987).
For HPV 6b; 7079 refers to the nucleotide position in the L1 region that is changed from the prototype sequence (Schwarz *et al.*, 1983).

FIGURE 32: A COMPARISON OF HPV 18 VARIANTS FOR THE L1 REGION (SAMPLES #1146/#3042)

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FIGURE 33: A COMPARISON OF HPV 6 VARIANTS FOR THE L1 REGION (SAMPLES #1175/#F114)

F114 TAGAAAACTTTTCTTTTAAATTAACCTCCCAAAAACTAAGGTTCTTATAGGGATCTGGCTTTTGCTTTTCAGGAGTGGGCTTTTGACAGGT
1175 TAGAAAACTTTTCTTTTAAATTAACCTCCCAAAAACTAAGGTTCTTATAGGGATCTGGCTTTTCCTTTTCAGGAGTGGGCTTTTGACAGGT

AATGGCCTGTGACTGCACATACCTATAGGTATCTTCTAATGTACCATTTGGGGGAGGCGATAACCCAAAGTTCCAGTCTTCCAAAACAGA
AATGGCCTGTGACTGCACATACCTATAGGTATCTTCTAATGTACCATTTGGGGGAGGCGATAACCCAAAGTTCCAGTCTTCCAAAACAGA

GGGATTCATTGTGTGAATATAGGCCATTACTTCAGCAGACAATGTAATGCTACATAATTGAAAAATAAATTGTAAATCATACTCTTCCAC
GGGATTCATTGTGTGAATATAGGCCATTACTTCAGCAGACAATGTAATGCTACATAATTGAAAAATAAATTGTAAATCATACTCTTCCAC

ATGACGCATGTACTCTTTATAATCAGAATTGGTGTATGTGGAAGATGTAGTTACGGATGCACATAATGTCATGTTGGTACTGCGTGTGGT
ATGACGCATGTACTCTTTATAATCAGAATTGGTGTATGTGGAAGATGTAGTTACGGATGCACATAATGTCATGTTGGTACTGCGTGTGGT

ATCTACCACAGTAACAAACAGTTGATTACCCCAACAAATACCATTGTTATGACCCTGGGC
ATCTACCACAGTAACAAACAGTTGATTACCCCAACAAATACCATTGTTATGACCCTGGGC

HOMOLOGY = 99.8%

• represents a nucleotide change between sequences
Underlined sequence is primer

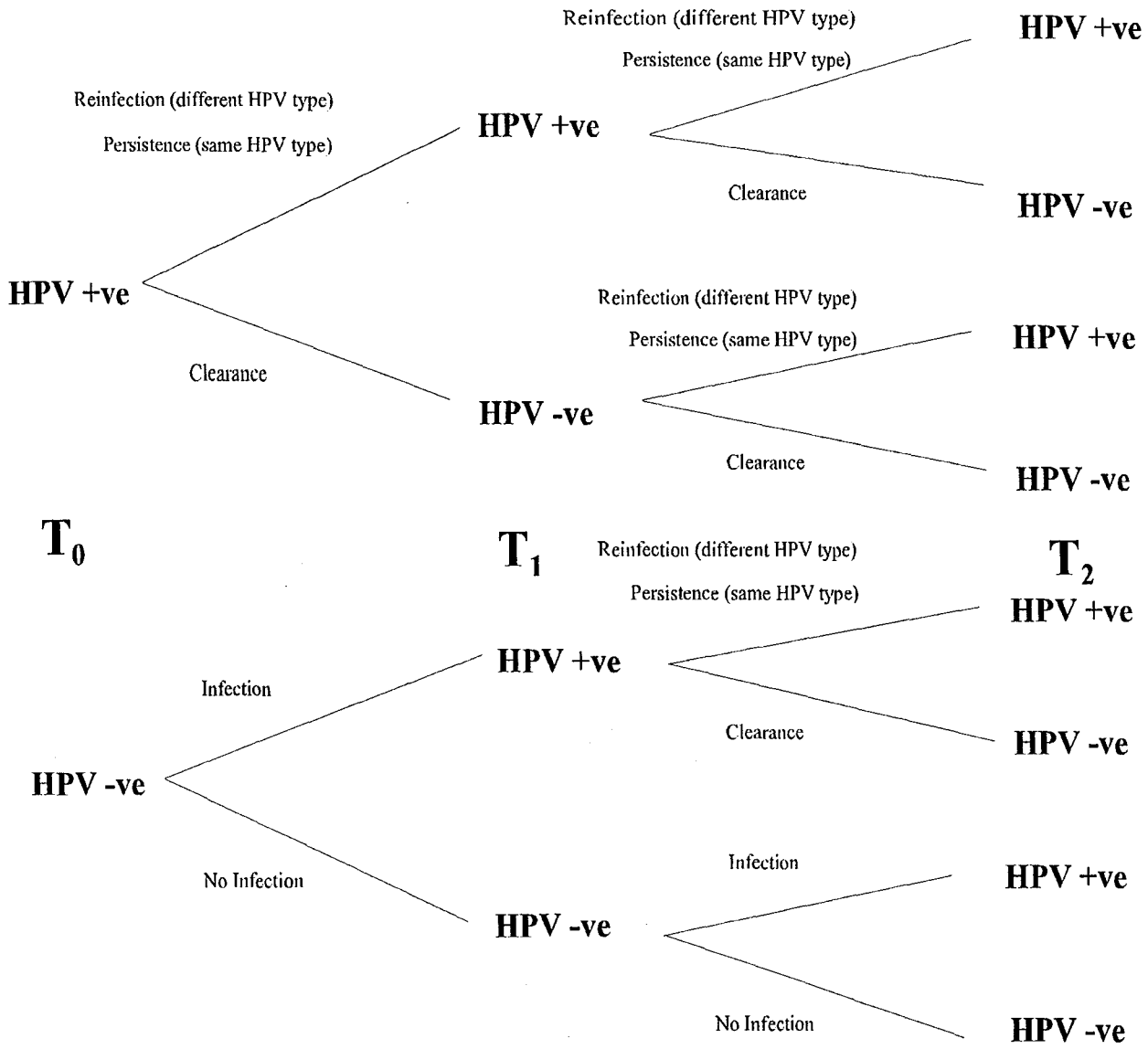
DISCUSSION

Issues of viral persistence or clearance are important in the context of disease risk, treatment and characterization of effective immunity. Repeated detection of HPV DNA over time may either be defined as persistent infection with the initial HPV type or clearance of the infection followed by reinfection; assuming of course, a minimal effect of sampling error. HPV genotyping is able to distinguish between persistence and reacquisition of virus when different genotypes are identified in consecutive samples. When the same genotype is identified over time, it may be referred to as HPV type-specific persistent infection by some definitions (Moscicki *et al.*, 1993; Hildesheim *et al.*, 1994; Evander *et al.*, 1995; Ho *et al.*, 1995; ter Harmsel *et al.*, 1999). Most studies establish persistence at only two points in time, but there is a great deal of variability in the time intervals chosen for sampling.

We have struggled with several issues. When the same genotype is detected on sequential visits, HPV typing alone may not distinguish between acquisition of a new infection with the same genotype from the same or a different sex partner or the alternative which is persistence of the original infection. Testing women at frequent intervals of time after initial identification of HPV infection, when combined with information about the woman's partners may be informative. A summary of the issues inherent in this discussion is presented in Figure 34.

FIGURE 34: INFECTION, CLEARANCE, REINFECTION AND PERSISTENCE OF HPV DNA

This flowchart summarizes HPV detection over time, assuming a minimal effect of sampling and typing errors. T_0, T_1 and T_2 represent three consecutive points in time for different samples in a patient.


Reinfection (different HPV type)
Persistence (same HPV type)
HPV +ve
HPV +ve
Clearance
HPV -ve
Reinfection (different HPV type)
Persistence (same HPV type)
HPV +ve
HPV +ve
Clearance
HPV -ve
Reinfection (different HPV type)
Persistence (same HPV type)
HPV +ve
HPV +ve
Clearance
HPV -ve
Infection
HPV -ve
No Infection
Infection
HPV +ve
HPV -ve
No Infection
HPV -ve
T0
T1
T2

Franco *et al.* (1994) have argued that identifying individual molecular variants in specimens may provide insight into these issues. Molecular variants of HPV can be identified on the basis of mutational patterns in the HPV DNA. If the variants in sequential isolates are identical, it is classified by Franco *et al.* as HPV variant-specific persistent infection. This may help to distinguish a new infection with the same genotype from persistence of the same genotype when the HPV variants are not identical.

Although mutations of an HPV variant during the replication of the virus in a person are possible, several groups suggest that this is unlikely (Ho *et al.*, 1991, 1993a, 1993b; Chan *et al.*, 1992; Xi *et al.*, 1995). Chan *et al.* (1992) show that there are very few differences in the HPV 16 LCR segment within a geographic region compared to the large diversity between regions. This seems to be consistent with a slow accumulation of mutations over time and therefore suggests that mutations occur rarely in HPVs such that a viral variant will not change over several years in an individual.

Numerous studies have focussed on the persistence of HPV 16 and several groups have shown nucleotide sequence variation in viral genes. In particular, some groups have shown many mutations in the long control region of HPV 16 (Ho *et al.*, 1991, 1993a, 1993b; Chan *et al.,* 1992; Xi *et al.,* 1993, 1995; Franco *et al.,* 1994; van Belkum *et al.,* 1995). Therefore, we hypothesized that sequencing of this region to examine HPV 16 persistence would constitute the first step in delineating the number and frequency of HPV 16 alleles in our population.

Our Study

We have examined samples which were found to be infected with HPV 16 by ethidium bromide and/or Amplicor strips on subsequent visits (Table 5). First annual samples are from a follow-up visit approximately one year after the initial cervical specimen was taken (baseline) and the second annual sample was taken approximately two years after the initial visit. HPV 16 studies on persistence concentrate on variants in the LCR region. Commonly, a 404bp region representing approximately 5% of the total genome is used. The long control region rather than other regions is examined because genomic sequences are more divergent in this region; long control regions differ dramatically between genotypes but have similar function (Ho *et al.*, 1991, 1993a; Chan *et al.*, 1992; Xi *et al.*, 1995). We sequenced an LCR fragment of the HPV 16 positive samples using HPV 16 type-specific primers (Franco *et al.*, 1994). The samples that were sequenced from the six patients in the LCR region are summarized in Table 6.

In every case examined, HPV types 16, 18, 6 and 66, were identical in LCR or L1 gene sequence in sequential isolates from a single individual. We expected to see changes in samples but did not observe any.

In a study by Xi *et al.* (1995), cloned PCR products showed that a predominant HPV 16 variant was present over time. Several explanations could account for this. It is possible that the initial HPV 16 infection is the one that establishes persistence. A second possibility is that certain HPV 16 variants have a biological advantage in a host. A variety of host factors can also potentially influence the ability of a particular variant to

become established; other sexually transmitted diseases such as HSV (DiPaolo *et al.*, 1990; Park *et al.*, 1995) and the status of the immune system with respect to influences or viral clearance (Xi *et al.*, 1995). Further investigations will have to be done to understand biologic behaviour differences, if any, of the HPV 16 variants since sequence characterizations have not been extensively related to biology. The virologic and clinical significance of the different variants is largely unknown although differences in E6 and E7 biology have been noted (Barbosa *et al.*, 1990; Stöppler *et al.*, 1996). Therefore, the significance of persistence infections by individual variants concerning risk of disease to a person is also unknown. Although there is no correlation between different variants of HPV 16 and disease, this does not formally eliminate the possibility of differences in oncogenic potential for HPV 16 variants.

Xi *et al.* (1997) speculated that HPV 16 variants might have different cellular tropisms. Some cell types may be more permissive for viral replication and therefore overgrowth of a particular variant may occur. Xi *et al.* (1995) have shown that the predominant variant in one person is the minor variant in others. However, these variants do not have an inherent growth advantage over other variants since in some patients they do not become the dominant variant.

Characterizations of minor variants is problematic. Different methods used will influence the results regarding mixed HPV 16 infections. Sequencing cloned PCR products may allow a large number of variants to be found, but unless a large number of clones are sequenced a less abundant variant may be missed (Xi *et al.* 1995). Direct

sequencing from the PCR product may also miss a variant that is very minor due to preferential amplification of the HPV 16 variant that is most abundant. A long term study has not been done with patients who have multiple HPV 16 variants to determine whether the ratio of variants fluctuates with time.

HPV 16 Long Control Region Alleles

In this study of six patients, we have seen only two LCR alleles for HPV 16 (Table 7). Most studies have illustrated that many nucleotides differ between isolates, for example at position 7518 (Ho *et al.*, 1991, 1993a, 1993b; Chan *et al.*, 1992; Bavin *et al.*, 1993; Smits *et al.*, 1994; Yamada *et al.*, 1995, 1997; Xi *et al.*, 1995, 1997) but few mutations have been found only once and most nucleotides in this region are conserved.

van Belkum *et al.* (1995) examined both promiscuous and monogamous women for HPV 16 variants. They found a large percentage of prototypes in the sequenced LCR region. Ho *et al.* (1993a) sequenced 301 LCR isolates from countries worldwide and found that 102 of them had only one nucleotide change at 7518, a guanine to an adenine, when compared to the prototype. Sixty of these isolates were identical to the prototype. Several other groups show that A_{7518} is the most common HPV 16 variant found (Appendix, Table 4). One must consider whether these are indeed the most common HPV 16 variants or whether any of these variants specifically cause persistence.

We have combined the HPV 16 variants and subtype sequences for the long control region analysed in the present study and from published studies in order to

attempt to determine the frequency of individual HPV LCR alleles (Appendix, Table 4) . The frequencies of all the LCR alleles are shown in Table 5 of the Appendix. The data is consistent with our study. The HPV LCR 16 allele, with one nucleotide change at 7518, is found 32.46% of the time. This is the most common variant. The HPV 16 prototype is found 19.09% of the time and is the second most common HPV 16 found in the long control region.

Obviously, then, one must consider the frequency of variants (alleles) when examining HPV 16 persistence. If it is a rare variant, it is probable that reinfection by another partner is less likely than persistence. However, reinfection by the same partner is a possibility. Perhaps it is not persistence at all and the most common variant is being detected over time because the woman is continuously being reinfected with this variant through the same or different partners. This is of higher probability for alleles which are more frequent in the population.

HPV Genotypes Other Than HPV 16

In contrast to HPV 16, the persistence of other HPV genotypes has not been extensively examined but persistence has been shown to be higher among women infected with high risk types compared to low risk types (Moscicki *et al.*, 1992, 1998; Hildesheim *et al.*, 1994; Evander *et al.*, 1995; Ho *et al.*, 1995; Brisson *et al.*, 1996; Chua *et al.*, 1996). We examined two patients infected with HPV 18, a high risk type, on subsequent occasions and two patients with HPV 6, a low risk HPV type, and a HPV 66

isolate of undetermined risk.

Franco *et al.* (1994) compared isolates from the same patient on subsequent occasions by sequencing the 450bp L1 region although no data was published. We used the same protocol by sequencing the L1 region of HPV isolates of patients, using Manos primers (Manos *et al.*, 1989), for infections with HPV 66, HPV 18 and HPV 6 as determined by ethidium bromide and/or Amplicor strips on subsequent visits (Table 8).

Same patient samples from separate visits were sequenced and aligned to determine whether the sequences were identical in the L1 region. Baseline sample #973 and first annual sample #2920 sequences are HPV 66 and aligned in Figure 26. This figure also shows baseline sample #1146 aligned to second annual sample #3299. These two sequences are HPV 18 positive. Also HPV 18, first annual sample #1957 and second annual sample #3042 sequences are aligned in Figure 27. Figure 28 shows baseline sample #1175, first annual sample #2392 and second annual sample #3157 aligned to each other. These samples are HPV 6 positive. All of these sequences from the baseline samples are identical to their respective first and second annual samples in the sequenced L1 region. Therefore, we found no differences between low and high risk HPV genotypes with respect to persistence. All the samples that we looked at showed that persistence is a strong possibility. Of course, reinfection from the same or different partners cannot be ruled out. The same considerations as those discussed for HPV 16 are still relevant.

Samples #2516 and #568 were sequenced but sequence data reveals that there is

more than one template present in these samples (Table 9). In the future, the PCR products will have to be cloned to ascertain whether there is an infection with multiple HPV genotypes and/or variants. Amplicor strip data shows that sample #2516 is infected with three HPV genotypes whereas sample #568 is infected with four genotypes. It is interesting that sample #F114, also infected with three HPV genotypes according to the Amplicor strip data, showed only HPV 6 when sequenced. PCR amplification of only one of the types occurred. HPV 6 was most likely present in a higher copy number compared to the other genotypes in this sample. On the other hand, it is plausible that all the HPV genotypes were present in low copy numbers in samples #568 and #2516.

In comparison to the HPV 66 prototype (unpublished, http://hpv-web.lanl.gov/), sample #973 has seven nucleotide changes, with a homology of 98.2% (Figure 29). Sample #1146 is 99.5% identical to the HPV 18 prototype (Cole *et al.*, 1987), having only two nucleotide changes (Figure 29). The other HPV 18 positive sample #1957, is less homologous to the prototype at 98.0% (Figure 30). The two HPV 6 positive samples, #1175 and #F114 show high homologies to the HPV 6b prototype (Schwarz *et al.*, 1983). Sample #1175 is identical to the prototype while sample #F114 has 99.8% homology to HPV 6b (Figures 30 and 31).

L1 Alleles of HPV 6, 18 and 66

L1 alleles for these three HPV genotypes that we studied are summarized in Table 10. Tables, similar to the table described earlier for HPV 16, based on a number of

studies with respect to variants and subtypes were also completed for genotypes of HPV 18, 6 and 66 (Appendix, Tables 6, 8 and 10).

We found two HPV 18 variants in four samples. When the L1 sequences from Table 6 of the Appendix were analysed to determine the frequency of HPV 18 L1 alleles, it was found that the two alleles that we identified are quite common (Appendix, Table 7). IS002 has nucleotide changes at 6625 and 6842 with respect to the prototype and is found in 55% of the HPV 18 L1 variants. IS168, which has eight nucleotide changes at 6579, 6581, 6625, 6626, 6719, 6749, 6842 and 6917, is found 12% of the time. This latter variant is less common than the prototype. The two HPV 18 variants, #1146 and #1957, are compared in Figure 32.

We found one HPV 6 variant in one sample since three of our samples contained the HPV 6b prototype. When the L1 sequences from Table 8 of the Appendix were combined, there were no HPV 6b prototype L1 strains found (Appendix, Table 9). This may be that HPV 6b is not very common, although our study suggests otherwise, or that the L1 variants came from only two studies suggesting that not enough data is available (Icenogle *et al.*, 1991; Caparrós-Wanderley *et al.*, 1999). Furthermore, the one variant that we found had one nucleotide change at 7079 of the HPV 6b prototype. The study by Caparrós-Wanderley *et al.* (1999) showed that a number of variants have this nucleotide change at 7079 but none of the isolates had only this one nucleotide change. In Figure 33, samples #1175 and #F114 are compared and this change is shown.

HPV 66 is an HPV genotype that has not been extensively studied. We found one

HPV 66 variant that was present in two samples. Only two other variants were found in published studies (Appendix, Table 10). None of the seven nucleotide changes found in this HPV 66 variant match published nucleotide changes. The frequency of the L1 region strains of HPV 66 cannot be determined since, in one of the cases, the number of strains is unknown. However, type-specific prevalence of HPV 66 (Appendix, Tables 1, 2 and 3) show that this genotype can be found in anywhere from 1-7.3% of HPV positive samples.

Two more tables of HPV variants and subtypes were compiled for HPV 57 (Appendix, Table 11) and 58 (Appendix, Table 12) to demonstrate that very few published studies have been done to determine different HPV strains. For each of these genotypes, only one study was published (Chan *et al.*, 1997; Stewart *et al.*, 1996). This seems unusual given that in the HPV type-specific studies examined, including our laboratory (Appendix, Tables 1, 2 and 3), HPV 58 is found in a significant proportion of HPV positive samples (1.2-11.1%). HPV 57, however, was not found in any HPV positive cervical samples. This genotype is more common in cutaneous lesions. Therefore, to consider the frequency of variants in a number of HPV types and to apply it in the context of persistence is difficult, if not impossible.

Conclusions

We found that persistence may be common in the context of either of the two definitions of persistence, both HPV type-specific and HPV variant-specific persistence.

There still remains several issues that need to be investigated in the context of persistent infection. Many studies have indicated that a woman persistently infected with HPV 16 develops CIN which is severe and often high grade (Koutsky *et al.*, 1992; Hildesheim *et al.*, 1994; Ho *et al.*, 1995; Remmink *et al.*, 1995; Chua *et al.*, 1996; Londesborough *et al.*, 1996; Moscicki *et al.*, 1998; ter Harmsel *et al.*, 1999). However, studies need to examine the individual variants of HPV 16 with respect to risk of disease and biologic behaviour differences.

Although HPV 6, HPV 18 and HPV 66 positive samples point to persistence, no other studies have been done to address the issue of variant-specific persistence of genotypes other than HPV 16. Therefore, it is difficult to make any firm conclusions regarding these genotypes and persistence. Much work needs to be done in this area.

There are a few significant considerations when the issue of persistence is analysed and particularly, the persistence of HPV 16. The most important is the commonality or rarity (frequency) of the particular HPV 16 variant. This brings a different perspective to persistence. We detected the two most common HPV 16 LCR variants which may suggest that persistence is not occurring and that these women are being infected continuously with these individual variants. Detection of an extremely rare variant on subsequent occasions would point more strongly to persistence. Perhaps neither of these two definitions of persistence is appropriate. In addition, one must also take into account the methodologies used for the detection of the variants and the sexual history of the patient. Furthermore, long term studies need to investigate persistence of

variants more thoroughly at frequent intervals.

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APPENDIX

TABLE 1: HPV TYPE-SPECIFIC PREVALENCE FROM SEVERAL STUDIES

REFERENCE HPV TYPES	*OUR LABORATORY*	Bauer *et al.* (1991) Journal of the American Medical Association; 265(4), 472-7	de Roda Husman *et al.* (1994) International Journal of Cancer, 56, 802-6	Remmink *et al.* (1995) International Journal of Cancer, 61, 306-11	Bosch *et al.* (1995) Journal of the National Cancer Institute, 87(11), 796-802
2	ND	ND	ND	ND	ND
6/11	HPV 6 = 45 (8.2%) HPV 11 = 7 (1.3%)	16 (5.3%)	HPV 6 = 20 (2.1%) HPV 11 = 9 (0.9%)	HPV 6 = 5 (2.2%) HPV 11 = 1 (0.4%)	HPV 6 = 1 (0.1%) HPV 11 = 1 (0.1%)
13	ND	ND	0	0	ND
16	108 (19.6%)	40 (13.2%)	442 (45.6%)	96 (42.3%)	465 (51.6%)
18	38 (6.9%)	24 (7.9%)	88 (9.2%)	18 (7.9%)	128 (14.2%)
26	1 (0.2%)	ND	ND	ND	4 (0.4%)
30	ND	ND	0	0	ND
31	31 (5.6%)	22 (7.3%)	99 (10.2%)	22 (9.7%)	49 (5.4%)
32	ND	ND	0	0	ND
33	15 (2.7%)	12 (4.0%)	52 (5.4%)	12 (5.3%)	26 (2.9%)
34	ND	ND	ND	ND	ND

35	3 (0.5%)	2 (0.7%)	7 (0.7%)	1 (0.44%)	16 (1.8%)
39	17 (3.1%)	15 (4.9%)	1 (0.1%)	5 (2.2%)	14 (1.5%)
40	1 (0.2%)	ND	1 (0.1%)	0	0
42	3 (0.5%)	ND	3 (0.3%)	1 (0.44%)	0
43	ND	ND	4 (0.4%)	0	ND
44	ND	ND	0	1 (0.44%)	ND
45	12 (2.2%)	5 (1.6%)	13 (1.3%)	1 (0.44%)	78 (8.6%)
51	29 (5.2%)	13 (4.3%)	14 (1.4%)	3 (1.3%)	7 (0.8%)
52	23 (4.2%)	21 (6.9%)	21 (2.2%)	7 (3.1%)	25 (2.8%)
53	45 (8.2%)	ND	0	0	0
54	27 (4.9%)	ND	5 (0.5%)	4 (1.8%)	0
55	3 (0.5%)	ND	1 (0.1%)	0	2 (0.2%)
56	12 (2.2%)	ND	2 (0.2%)	1 (0.44%)	16 (1.8%)
57	0	ND	0	0	ND
58/PAP251	32 (5.8%)	11 (3.6%)	21 (2.2%)	10 (4.4%)	19 (2.1%)
59	14 (2.5%)	ND	7 (0.7%)	1 (0.44%)	15 (1.7%)
61	ND	ND	3 (0.3%)	2 (0.88%)	ND

62	ND	ND	ND	ND	ND
64	ND	ND	ND	ND	ND
66/PAP88	34 (6.2%)	22 (7.3%)	10 (1.0%)	2 (0.88%)	0
67	ND	ND	ND	ND	ND
68/ME180	3 (0.5%)	ND	ND	ND	11 (1.2%)
69	ND	ND	ND	ND	ND
70	ND	ND	ND	ND	ND
72	ND	ND	ND	ND	ND
MM4/W13B	5 (0.9%)	ND	ND	ND	6 (0.7%)
MM7/PAP291	8 (1.4%)	ND	ND	ND	1 (0.1%)
MM8/PAP155	26 (4.7%)	17 (5.6%)	ND	ND	0
MM9/PAP238A /HPV 73	10 (1.8%)	ND	ND	ND	6 (0.7%)
W13A	ND	22 (7.3%)	ND	ND	ND
AE2	ND	ND	ND	ND	ND
AE6	ND	ND	ND	ND	ND
AE7	ND	ND	ND	ND	ND
AE8	ND	ND	ND	ND	ND

UNKNOWN HPV TYPES	-----	61 (20.1%)	146 (15.1%)	34 (15.0%)	12 (1.3%)
STUDY INFORMATION	Patients from University of Toronto Health Service clinic; PAP smears; **552** HPV positive samples; 129 samples with more than one HPV type (93-2 types; 25-3 types; 8-4 types; 1-5 types; 2-6 types); Amplicor line blot assay (MY09/11)	Patients from University Health Service, University of California; routine examination; cervical swabs; **303** HPV positive samples (213 patients); 60 samples with more than one HPV type (39-2 types; 14-3 types; 5-4 types; 2-5 types); consensus (MY09/11) PCR + dot blot hybridization	Patients attending outpatient clinics of hospitals in Amsterdam, The Netherlands; cervical scrapes; **969** HPV positive samples; 85 samples with more than one HPV type; GP-PCR (GP5/6) + hybridization assay	Patients attending an outpatient clinic in a hospital for abnormal pap smears in Amsterdam, The Netherlands; cervical smears; **227** HPV positive samples (213 patients); 14 samples with more than one HPV type (all double infections; GP-PCR (GP5/6) + hybridization assay	Invasive cervical cancer patients from 32 hospitals and 22 countries worldwide; tumour specimens; **902** HPV positive samples; 36 samples with more than one HPV type (all double infections); consensus PCR (MY09/11) + hybridization assay

TABLE 2: HPV TYPE-SPECIFIC PREVALENCE FROM SEVERAL STUDIES

REFERENCE HPV TYPES	Franco *et al.* (1995) Journal of Infectious Diseases, 172, 756-63	Londesborough *et al.* (1996) International Journal of Cancer, 69, 364-8	Burk *et al.* (1996) Journal of Infectious Diseases, 174, 679-89 Note : Data presented as percentages	Ho *et al.* (1998a) New England Journal of Medicine, 338(7), 423-8 Note : Data presented as percentages
2	ND	ND	0	ND
6/11	HPV 6 = 11 (8.3%) HPV 11 = 12 (9.0%)	ND	≤5% (for each)	HPV 6 = 5% HPV 11 = <2%
13	ND	ND	0	ND
16	28 (21.1%)	45 (31.7%)	23 (14.7%)	7%
18	11 (8.3%)	9 (6.3%)	12 (7.6%)	4%
26	0	ND	≤5%	<2%
30	ND	ND	ND	ND
31	3 (2.2%)	35 (24.7%)	≤5%	2%
32	ND	ND	≤5%	<2%
33	13 (9.8%)	13 (9.2%)	≤5%	2%
34	ND	ND	0	<2%
35	4 (3.0%)	5 (3.5%)	≤5%	2%

39	0	ND	9 (5.7%)	3%
40	0	ND	≤5%	<2%
42	0	ND	0	<2%
43	ND	ND	ND	ND
44	ND	ND	ND	ND
45	3 (2.2%)	2 (1.4%)	8 (5.1%)	2%
51	0	ND	9 (5.7%)	8%
52	4 (3.0%)	2 (1.4%)	≤5%	3%
53	5 (3.7%)	ND	14 (8.9%)	3%
54	1 (0.75%)	ND	≤5%	2%
55	0	ND	≤5%	<2%
56	2 (1.5%)	ND	≤5%	<2%
57	0	ND	0	ND
58/PAP251	7 (5.3%)	8 (5.6%)	8 (5.1%)	3%
59	0	ND	10 (6.4%)	4%
61	ND	ND	9 (5.7%)	3%
62	ND	ND	0	ND

64	ND	ND	0	ND
66/PAP88	7 (5.3%)	ND	8 (5.7%)	7%
67	ND	ND	0	<2%
68/ME180	1 (0.75%)	ND	≤5%	<2%
69	ND	ND	0	0
70	ND	ND	≤5%	<2%
72	ND	ND	0	0
MM4/W13B	ND	ND	0	<2%
MM7/PAP291	ND	ND	≤5%	3%
MM8/PAP155	ND	ND	11 (7.0%)	7%
MM9/PAP238A /HPV73	ND	ND	9 (5.7%)	3%
W13A	ND	ND	ND	ND
AE2	ND	ND	0	<2%
AE6	ND	ND	ND	<2%
AE7	ND	ND	ND	3%
AE8	ND	ND	ND	<2%

UNKNOWN HPV TYPES	21 (15.8%)	23 (16.2%)	33 (21.0%) Note: 46 (29.3%) is made up of all the types with ≤5%. Percentages total >100% because of samples with multiple genotypes	-----
STUDY INFORMATION	Patients from cervical cancer screening program, Joao Pessoa, Brazil; ecto and endo cervical specimens; **133** HPV positive samples (96 patients); 26 samples with more than one HPV type (19-2 types; 3-3 types; 4-4 types); consensus PCR (MY09/11) + dot blot hybridization	Patients with 2 abnormal pap smears; Whittington Hospital, London, England; cervical scrapes; **142** HPV positive samples; consensus PCR (MY09/11) + SHARP hybridization assay (HPV type-specific RNA probes)	Patients are 157 students attending the state university from New Jersey; cervicovaginal lavage samples; **209** HPV positive samples; 34 samples with more than one HPV type; consensus PCR (MY09/11) + southern blot hybridization	Patients are 175 students attending university in New Brunswick, New Jersey; cervicovaginal lavage samples; **unknown** number of samples; consensus PCR (MY09/11) + southern blot hybridization

TABLE 3: HPV TYPE-SPECIFIC PREVALENCE FROM SEVERAL STUDIES

REFERENCE HPV TYPES	Ho *et al.* (1998b) International Journal of Cancer, 78, 281-5 Note : Data presented as percentages	Lombard *et al.* (1998) Journal of Clinical Oncology, 16(8), 2613-9	Gravitt *et al.* (1998) Journal of Clinical Microbiology, 36(10), 3020-7	Coutlée *et al.* (1999) Journal of Clinical Microbiology, 37(6), 1852-7
2	ND	ND	ND	ND
6/11	HPV 6 = 5.7% HPV 11 = ≤5 patients	0	6 (3.8%) (probes pooled as pairs)	HPV 6 = 14 (6.0%) HPV 11 = 1 (0.4%)
13	ND	ND	ND	ND
16	23.3%	150 (60.7%)	22 (13.8%)	7 (3.0%)
18	7.6%	31 (12.6%)	7 (4.4%)	9 (3.8%)
26	≤5 patients	ND	HPV 26 or MM8 = 5 (3.1%) (probes pooled as pairs)	0
30	ND	ND	ND	ND
31	9.2%	3 (1.2%)	12 (7.5%)	13 (5.6%)
32	≤5 patients	ND	ND	ND
33	6.1%	6 (2.4%)	2 (1.2%)	8 (3.4%)
34	0	ND	ND	ND

35	5.3%	1 (0.4%)	1 (0.6%)	5 (2.1%)
39	4.6%	0	10 (6.3%)	18 (7.7%)
40	≤5 patients	ND	0	0
42	1.9%	0	0	0
43	ND	ND	ND	ND
44	ND	ND	ND	ND
45	4.6%	1 (0.4%)	4 (2.5%)	7 (3.0%)
51	5.0%	ND	13 (8.1%)	13 (5.6%)
52	11.8%	1 (0.4%)	15 (9.4%)	15 (6.4%)
53	6.5%	ND	13 (2.5%)	20 (8.6%)
54	3.4%	ND	7 (4.4%)	14 (6.0%)
55	≤5 patients	ND	2 (1.2%)	4 (1.7%)
56	8.0%	ND	9 (5.6%)	24 (10.3%)
57	ND	ND	0	0
58/PAP251	11.1%	3 (1.2%)	5 (3.1%)	12 (5.1%)
59	1.5%	ND	7 (4.4%)	3 (1.3%)
61	4.2%	ND	ND	ND

62	ND	ND	ND	ND
64	ND	ND	ND	ND
66/PAP88	4.2%	ND	7 (4.4%)	12 (5.1%)
67	≤5 patients	ND	ND	ND
68/ME180	2.3%	ND	3 (1.9%)	5 (2.1%)
69	≤5 patients	ND	ND	ND
70	3.1%	ND	ND	ND
72	≤5 patients	ND	ND	ND
MM4/W13B	0	ND	1 (0.6%)	0
MM7/PAP291	3.1%	ND	6 (3.8%)	16 (6.8%)
MM8/PAP155	≤5 patients	ND	HPV 26 or MM8 = 5 (3.1%) (probes pooled as pairs)	11 (4.7%)
MM9/PAP238A /HPV73	3.8%	ND	3 (1.9%)	3 (1.3%)
W13A	ND	ND	ND	ND
AE2	≤5 patients	ND	ND	ND
AE6	0	ND	ND	ND
AE7	3.1%	ND	ND	ND

AE8	3.1%	ND	ND	ND
UNKNOWN HPV TYPES	10.3%	51 (20.7%)	-----	-----
STUDY INFORMATION	Patients with abnormal pap smears attending health care clinics, Albert Einstein College of Medicine; cervicovaginal lavage samples; **262** HPV positive samples; consensus PCR (MY09/11) + southern blot hybridization	Invasive cervical carcinoma patients attending the Institut Curie, Paris, France; tumour tissues; **247** HPVpositive samples; 1 sample with more than one HPV type; L1 consensus PCR + southern blot hybridization	Patients are students attending the University of New Mexico Health Sciences Center; cervical specimens; **160** HPV positive samples; 15 samples with more than one HPV type; dot blot + Amplicor line blot assay (MY09/11)	Genital specimens were from cohort studies; cervicovaginal lavage specimens from Canadian Women's HIV Study; **234** HPV positive samples; 46 samples with more than one HPV type; Amplicor line blot assay (MY09/11)

TABLE 4: HPV 16 GENOTYPE, VARIANTS AND SUBTYPES

STRAIN NAME	LENGTH	SPECIFIC GENE(S)	NUCLEOTIDE POSITION CHANGES	% HOMOLOGY TO PROTOTYPE	PATIENT POPULATION	# PATIENTS STRAINS FOUND	SOURCE	REFERENCE
HPV 16, prototype	7904bp Note: 364bp region is from nucleotides 7477-7840	ALL	NA	NA	NA	NA	cervical carcinoma	Seedorf *et al.* (1985) Virology, 145, 181-5
prototype	364bp Note: LCR region adjusted 1bp to align sequence	LCR	none	100	patients from around the world; SA=South Africa; Z=Zaire; SL= Sierra Leone; SN = Senegal, AP= Pueblo Indian; AM= Amazonian Indian; S=Singapore; B=Brazil; T=Tanzania, F= Finland; H= Greece; AR=Arabia; AC= Argentina; NY= New York; D= Detroit; IND=India; J=Japan; E=Greenland; AN= Navajo Indian; NC= North Carolina; P=Peru; G=Germany	60/301 (5AN, 1AP, 13S, 1SN, 13B, 2F, 1T, 5IND, 2G, 2E, 4SA, 1AC, 3H, 1NY, 1D, 5NC)	most from cervical smears, biopsy specimens from cervical carcinoma and biopsy specimens from CIN I, II, III, 18 male patients-punch biopsy of penis	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23; Chan *et al.* (1992) Journal of Virology, 66(4), 2057-66; Ho *et al.* (1991) Journal of Clinical Microbiology, 29(9), 1765-72

S-5	364bp	LCR	7664	99.7	patients from around the world	2/301 (1S, 1B)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
S-2 (Caski)	364bp	LCR	7518	99.7	patients from around the world	102/301 (9AP, 4AN, 5AM, 1J, 2AC, 2H, 5NY, 1AR, 1D, 13IND, 3NC, 1SL, 4F, 17S, 8B, 3T, 12G, 2SA, 9P)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
S-13	364bp	LCR	7518, 7839	99.5	patients from around the world	3/301 (2S, 1B)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
B-24	364bp	LCR	7752, 7775	99.5	patients from around the world	1/301 (1B)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
B-3	364bp	LCR	7518, 7565	99.5	patients from around the world	1/301 (1B)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23

G-13	364bp	LCR	7518, 7710	99.5	patients from around the world	1/301 (1G)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
G-10	364bp	LCR	7518, 7549	99.5	patients from around the world	2/301 (2G)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
IND-7	364bp	LCR	7518, 7711(G)	99.5	patients from around the world	1/301 (1IND)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
IND-4	364bp	LCR	7518, 7663	99.5	patients from around the world	1/301 (1IND)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
IND-5	364bp	LCR	7518, 7789	99.5	patients from around the world	2/301 (1IND, 1NY)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
S-H17	364bp	LCR	7518, 7727	99.5	patients from around the world	2/301 (1S, 1AC)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23

AP-4	364bp	LCR	7518, 7779	99.5	patients from around the world	1/301 (1AP)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
SA-9	364bp	LCR	7493, 7518	99.5	patients from around the world	1/301 (1SA)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
S-H2a	364bp	LCR	7518, 7757	99.5	patients from around the world	16/301 (11S, 2SL, 3E)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
SL-6a	364bp	LCR	7518, 7711, 7761	99.2	patients from around the world	1/301 (1SL)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
S-7	364bp	LCR	7518, 7727, 7839	99.2	patients from around the world	5/301 (5S)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
J-3	364bp	LCR	7518, 7727, 7778, 7839	98.9	patients from around the world	16/301 (5J, 11S)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23

Ss-1a	364bp	LCR	7477, 7478, 7575, 7636	98.9	patients from around the world	1/301 (1S)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
S-14	364bp	LCR	7478, 7518, 7799, 7839	98.9	patients from around the world	1/301 (1S)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
G-21a	364bp	LCR	7518, 7549, 7622, 7770	98.9	patients from around the world	2/301 (2G)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
J-5	364bp	LCR	7518, 7727, 7756, 7778, 7839	98.6	patients from around the world	1/301 (1J)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
S-23	364bp	LCR	7486, 7518, 7711, 7761, 7783, 7831	98.4	patients from around the world	2/301 (1S, 1T)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
S-21	364bp	LCR	7482, 7486, 7518, 7666, 7761, 7783	98.4	patients from around the world	1/301 (1S)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23

T-8	364bp	LCR	7486, 7518, 7686, 7761, 7783, 7831	98.4	patients from around the world	9/301 (2T, 1NC, 2SL, 3SN, 1NY)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
T-4	364bp	LCR	7486, 7518, 7686, 7711, 7761, 7783, 7831	98.1	patients from around the world	8/301 (5T, 1SA, 1AR, 1NY)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
T-16	364bp	LCR	7486, 7518, 7666, 7686, 7761, 7783, 7831	98.1	patients from around the world	2/301 (1T, 1E)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
T-S6a	364bp	LCR	7486, 7518, 7556, 7686, 7711, 7761, 7783, 7831	97.8	patients from around the world	1/301 (1T)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
T-S6b	364bp	LCR	7486, 7518, 7645, 7686, 7711, 7761, 7783, 7831	97.8	patients from around the world	1/301 (1T)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
AN-10	364bp	LCR	7518, 7666, 7761, 7783, 7823, 7831, 7834, 7836	97.8	patients from around the world	1/301 (1AN)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23

AN-12	364bp	LCR	7482, 7486, 7518, 7666, 7786, 7726, 7761, 7783	97.8	patients from around the world	3/301 (3AN)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
IND-8	364bp	LCR	7482, 7486, 7518, 7666, 7686, 7726, 7740, 7761, 7783	97.5	patients from around the world	16/301 (2IND, 3S, 1NC, 1P, 9B)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
B-11	364bp	LCR	7482, 7486, 7518, 7666, 7686, 7726(T), 7740, 7761, 7783	97.5	patients from around the world	3/301 (2B, 1AM)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
B-14	364bp	LCR	7482, 7486, 7504, 7518, 7666, 7686, 7726, 7761, 7783	97.5	patients from around the world	1/301 (1B)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
P-1	364bp	LCR	7482, 7486, 7504, 7518, 7666, 7686, 7726(T), 7761, 7783	97.5	patients from around the world	1/301 (1P)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23

B-1	364bp	LCR	7482, 7486, 7518, 7666, 7686, 7726, 7740, 7761, 7783, duplicate of segment 7338-7550	97.5 (without duplicate)	patients from around the world	1/301 (1B)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
S-21a	364bp	LCR	7482, 7486, 7518, 7666, 7761, 7783, 7823, 7831, 7834, 7836	97.3	patients from around the world	4/301 (2S, 1AN, 1NY)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
SN-4	364bp	LCR	7482, 7486, 7518, 7666, 7686, 7761, 7783, 7823, 7834, 7836	97.3	patients from around the world	1/301 (1SN)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
T-S5	364bp	LCR	7482, 7486, 7518, 7640, 7686, 7761, 7783, 7823, 7831, 7834	97.3	patients from around the world	1/301 (1T)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
B-19	364bp	LCR	7482, 7486, 7504, 7518, 7666, 7686, 7726, 7740, 7761, 7783	97.3	patients from around the world	1/301 (1B)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23

J-1	364bp	LCR	7482, 7486, 7504, 7518, 7666, 7686, 7726(T), 7740, 7761, 7783	97.3	patients from around the world	1/301 (1J)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
T-S5a	364bp	LCR	7482, 7486, 7518, 7640, 7666, 7686, 7761, 7783, 7823, 7831, 7834	97.0	patients from around the world	1/301 (1T)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
T-1	364bp	LCR	7482, 7486, 7518, 7666, 7686, 7761, 7783, 7823, 7831, 7834, 7836	97.0	patients from around the world	11/301 (4T, 1SN, 2Z, 1SL, 3SA)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
T-S5b	364bp	LCR	7482, 7486, 7518, 7621, 7640, 7666, 7686, 7761, 7783, 7823, 7831, 7834	96.7	patients from around the world	1/301 (IT)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
T-S5c	364bp	LCR	7482, 7486, 7518, 7640, 7644, 7666, 7686, 7761, 7783, 7823, 7831, 7834	96.7	patients from around the world	1/301 (1T)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23

T-S3	364bp	LCR	7482, 7486, 7518, 7564, 7666, 7686, 7761, 7783, 7823, 7831, 7834, 7836	96.7	patients from around the world	1/301 (1T)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
T-S3b	364bp	LCR	7482, 7486, 7518, 7666, 7686, 7746, 7761, 7783, 7823, 7831, 7834, 7836	96.7	patients from around the world	1/301 (1T)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
T-S3c	364bp	LCR	7482, 7486, 7518, 7561, 7666, 7686, 7761, 7783, 7823, 7831, 7834, 7836	96.7	patients from around the world	1/301 (1T)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
T-S3a	364bp	LCR	7482, 7486, 7501, 7518, 7594, 7666, 7686, 7761, 7783, 7823, 7831, 7834, 7836	96.4	patients from around the world	1/301 (IT)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
B-8a	364bp	LCR	deletion 7535-7707	52.7	patients from around the world	1/301 (1B)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23

T-15	364bp	LCR	7486, 7518, 7666, 7686, 7761, 7783, 7831; deletion 7535-7707	50.8	patients from around the world	1/301 (1T)	cervical smears, biopsies from carcinomas and CINs, penile biopsies	Ho *et al.* (1993a) Journal of Virology, 67(11), 6413-23
OR.4997	364bp Note: LCR region adjusted 2bp to align sequence	LCR	7518	99.7	Patients From New Mexico and Oregon	9/32 (3/32 prototype)	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53; Wheeler *et al.* (1997) Journal of Clinical Microbiology, 35(1), 11-19
OR.2087	364bp	LCR	7518, 7727, 7839	99.2	Patients From New Mexico and Oregon	3/32	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.7574	364bp	LCR	7518, 7727, 7778, 7839	98.8	Patients From New Mexico and Oregon	1/32	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.8392	364bp Note: sequence begins at 7671	LCR	7686, 7726(T), 7749, 7783	98.8	Patients From New Mexico and Oregon	1/32	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.7587	364bp	LCR	7486, 7518, 7686, 7761, 7783, 7831	98.4	Patients From New Mexico and Oregon	3/32	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53

OR.1905	364bp	LCR	7486, 7518, 7686, 7711, 7761, 7783, 7831	98.1	Patients From New Mexico and Oregon	1/32	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.5691	364bp	LCR	7482, 7486, 7504, 7518, 7666, 7686, 7726, 7761, 7783	97.6	Patients From New Mexico and Oregon	2/32	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
NM.7529	364bp	LCR	7482, 7486, 7518, 7666, 7686, 7726, 7740, 7761, 7783	97.6	Patients From New Mexico and Oregon	4/32	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.4541	364bp	LCR	7482, 7486, 7518, 7666, 7686, 7726, 7740, 7749, 7761, 7783	97.3	Patients From New Mexico and Oregon	1/32	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.7145	364bp	LCR	7482, 7486, 7518, 7666, 7761, 7783, 7823, 7831, 7834, 7836	97.3	Patients From New Mexico and Oregon	1/32	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.3136	364bp	LCR	7482, 7486, 7518, 7666, 7686, 7726, 7761, 7783, 7823, 7831	97.3	Patients From New Mexico and Oregon	1/32	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53

OR.3473	364bp	LCR	7482, 7486, 7518, 7666, 7686, 7761, 7783, 7823, 7831, 7834, 7836	97.0	Patients From New Mexico and Oregon	2/32	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
IS.645	364bp Note: LCR region adjusted 3bp to align sequence	LCR	7518	99.7	Patients from 22 countries worldwide	6/28 (1/28 prototype)	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.105	364bp	LCR	7784	99.7	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.1032	364bp	LCR	7493, 7784	99.4	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.845	364bp	LCR	7486, 7518, 7686, 7761, 7783, 7831	98.4	Patients from 22 countries worldwide	2/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.393	364bp	LCR	7486, 7518, 7608, 7686, 7761, 7783, 7831	98.1	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72

IS.838	364bp	LCR	7486, 7518, 7686, 7761, 7783, 7789, 7831	98.1	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.347	364bp	LCR	7486, 7518, 7666, 7686, 7761, 7783, 7831	98.1	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS. 53	364bp	LCR	7482, 7486, 7504, 7518, 7666, 7686, 7726, 7761 7783	97.5	Patients from 22 countries worldwide	2/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.545	364bp	LCR	7482, 7486, 7518, 7666, 7686, 7726, 7740, 7761, 7783	97.5	Patients from 22 countries worldwide	2/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS. 42	364bp	LCR	7482, 7486, 7518, 7666, 7686, 7726, 7761, 7783, 7831	97.5	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.812	364bp	LCR	7482, 7486, 7518, 7666, 7686, 7761, 7783, 7823, 7834, 7836	97.3	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72

IS.170	364bp	LCR	7482, 7486, 7518, 7666, 7686, 7761, 7783, 7823, 7831, 7834	97.3	Patients from 22 countries worldwide	2/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.811	364bp	LCR	7482, 7486, 7518, 7666, 7667, 7686, 7761, 7783, 7823, 7831, 7834	97.0	Patients from 22 countries worldwide	2/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.177	364bp	LCR	7482, 7486, 7518, 7666, 7685, 7686, 7761, 7783, 7823, 7831, 7834	97.0	Patients from 22 countries worldwide	2/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.830	364bp	LCR	7482, 7486, 7518, 7640, 7666, 7686, 7761, 7783, 7823, 7831, 7834	97.0	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.846	364bp	LCR	7482, 7486, 7518, 7640, 7666, 7686, 7761, 7783, 7789(A), 7823, 7831, 7834	96.7	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72

Dt4	681bp Note: 681bp region is from nts 7170 to 7850 of prototype; LCR region adjusted 1bp to align sequence	LCR	7191	99.9	Patients from Barbados and Netherlands	3/19 (2/19 Prototype)	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62
Bt22	681bp	LCR	7518 (7191, 7230, 7231 not sequenced)	99.9	Patients from Barbados and Netherlands	1/19	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62
Dt42	681bp	LCR	7191, 7518	99.7	Patients from Barbados and Netherlands	5/19	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62
Dc207	681bp	LCR	7191, 7358, 7518	99.6	Patients from Barbados and Netherlands	1/19	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62
Bt7	681bp	LCR	7191, 7231, 7518	99.6	Patients from Barbados and Netherlands	3/19	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62

Bt9	681bp	LCR	7191, 7230, 7518	99.6	Patients from Barbados and Netherlands	4/19	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62
HHH66	460bp Note: 460bp region is from nts 7469 to 24 of prototype; LCR region adjusted 3bp (up to 7865) and 2bp to align sequence	LCR	7518	99.8	STD clinic patients and university students (Washington)	2/19 (3/19 prototype)	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802
HHH165	460bp	LCR	7493	99.8	STD clinic patients and university students (Washington)	1/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802
HHH260	460bp	LCR	7482	99.8	STD clinic patients and university students (Washington)	1/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802
HHH103	460bp	LCR	7518, 12	99.6	STD clinic patients and university students (Washington)	1/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802

HHH101	460bp	LCR	7518, 7603	99.6	STD clinic patients and university students (Washington)	2/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802
HHH105	460bp	LCR	7490, 7875	99.6	STD clinic patients and university students (Washington)	1/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802
HHH7	460bp	LCR	7494, 7518	99.6	STD clinic patients and university students (Washington)	2/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802
HHH228	460bp	LCR	7518, 7866, 12	99.3	STD clinic patients and university students (Washington)	1/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802
HHH81	460bp	LCR	7518, 7727, 7839, 24	99.1	STD clinic patients and university students (Washington)	1/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802
HHH5	460bp	LCR	7486, 7518, 7686, 7761, 7783, 7831, 7874	98.5	STD clinic patients and university students (Washington)	1/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802

HHH208	460bp	LCR	7486, 7518, 7666, 7686, 7726, 7761, 7783, 7884	98.3	STD clinic patients and university students (Washington)	1/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802
HHH301	460bp	LCR	7482, 7486, 7518, 7666, 7686, 7726, 7740, 7761, 7783, 7884	97.8	STD clinic patients and university students (Washington)	1/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802
HHH198	460bp	LCR	7482, 7486, 7518, 7633, 7666, 7761, 7783, 7823, 7831, 7834, 7836	97.6	STD clinic patients and university students (Washington)	1/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802
HDS85	386bp Note: 386bp region is from nts 7444-7829 of prototype; LCR region adjusted 1bp to align sequence	LCR	7518	99.7	STD clinic, Washington Note: 12 isolates total but only 4 patients; different samples; different times (1989-1993)	1/12 (5/12 prototype)	vulvar, vaginal and cervical swabs	Xi *et al.* (1995) The Journal of Infectious Diseases, 172, 747-55
HDS81a	386bp	LCR	7482, 7486, 7518, 7666, 7686, 7726, 7740, 7761	97.9	STD clinic, Washington	1/12	vulvar, vaginal and cervical swabs	Xi *et al.* (1995) The Journal of Infectious Diseases, 172, 747-55

HDS81a	386bp	LCR	7482, 7486, 7518, 7666, 7686, 7726, 7740, 7761, 7783	97.7	STD clinic, Washington	5/12	vulvar, vaginal and cervical swabs	Xi *et al.* (1995) The Journal of Infectious Diseases, 172, 747-55
HDS84-d	262bp Note: 262bp region is from nts 7747 to 104 of prototype; LCR region adjusted 1bp (up to 7904) to align sequence	LCR	12	99.6	STD clinic, Washington Note: 14 isolates total but only 4 patients; different samples; different times (1989-1993)	1/14 (6/14 prototype)	vulvar, vaginal and cervical swabs	Xi *et al.* (1995) The Journal of Infectious Diseases, 172, 747-55
HDS106-a	262bp	LCR	7839, 24	99.2	STD clinic, Washington	2/14	vulvar, vaginal and cervical swabs	Xi *et al.* (1995) The Journal of Infectious Diseases, 172, 747-55
HDS106-b	262bp	LCR	7866, 12	99.2	STD clinic, Washington	4/14	vulvar, vaginal and cervical swabs	Xi *et al.* (1995) The Journal of Infectious Diseases, 172, 747-55
HDS84-b	262bp	LCR	7789, 7866, 12	98.9	STD clinic, Washington	1/14	vulvar, vaginal and cervical swabs	Xi *et al.* (1995) The Journal of Infectious Diseases, 172, 747-55

CO	883bp Note: 883bp region is from nucleotides 7143-121; LCR region was adjusted to align sequence	LCR	7191, 7392, 7431, 7433, 7447, 7518, 7521; deletion of 7726 and 7861; insertion between nts 7460 and 7461	98.8	asymptomatic patient	NA	cervical scrapes	Bavin *et al.* (1993) Journal of Medical Virology, 39, 267-72
C3	883bp	LCR	7191, 7231, 7261, 7337, 7392, 7393, 7429, 7430, 7431, 7433, 7482, 7486, 7518, 7645, 7666, 7686, 7726, 7739, 7761, 7783, 7884; deletion of 7861; insertion between nts 7460 and 7461	97.5	patient diagnosed with CIN III	NA	cervical scrapes	Bavin *et al.* (1993) Journal of Medical Virology, 39, 267-72
4	419bp Note: 419bp region is from nts 7109 to 7527 of prototype	LCR	7314	99.8	STD clinic; male and female patients from the Netherlands	3/53 Note: 1 patient 2 separate times (27/53 prototype; 4 patients 2 separate times)	cervical smears for females; swabs from urethra for males	van Belkum *et al.* (1995) Journal of Clinical Microbiology, 33(11), 2957-62
5	419bp	LCR	7193	99.8	STD clinic; male and female patients from the Netherlands	1/53	cervical smears for females; swabs from urethra for males	van Belkum *et al.* (1995) Journal of Clinical Microbiology, 33(11), 2957-62

8	419bp	LCR	7191	99.8	STD clinic; male and female patients from the Netherlands	5/53 Note: 1 patient 2 separate times	cervical smears for females; swabs from urethra for males	van Belkum *et al.* (1995) Journal of Clinical Microbiology, 33(11), 2957-62
22	419bp	LCR	7447 (T or C)	99.8	STD clinic; male and female patients from the Netherlands	1/53	cervical smears for females; swabs from urethra for males	van Belkum *et al.* (1995) Journal of Clinical Microbiology, 33(11), 2957-62
20	419bp	LCR	7191 (7231 not sequenced)	99.8	STD clinic; male and female patients from the Netherlands	1/53	cervical smears for females; swabs from urethra for males	van Belkum *et al.* (1995) Journal of Clinical Microbiology, 33(11), 2957-62
13	419bp	LCR	(7191, 7192, 7193, 7231 not sequenced)	100	STD clinic; male and female patients from the Netherlands	4/53	cervical smears for females; swabs from urethra for males	van Belkum *et al.* (1995) Journal of Clinical Microbiology, 33(11), 2957-62
14	419bp	LCR	7447 (7191, 7192, 7193, 7231 not sequenced)	99.8	STD clinic; male and female patients from the Netherlands	3/53	cervical smears for females; swabs from urethra for males	van Belkum *et al.* (1995) Journal of Clinical Microbiology, 33(11), 2957-62

15	419bp	LCR	7314 (7191, 7192, 7193, 7231 not sequenced)	99.8	STD clinic; male and female patients from the Netherlands	2/53	cervical smears for females; swabs from urethra for males	van Belkum *et al.* (1995) Journal of Clinical Microbiology, 33(11), 2957-62
29	419bp	LCR	7358 (7191, 7192, 7193, 7231 not sequenced)	99.8	STD clinic; male and female patients from the Netherlands	1/53	cervical smears for females; swabs from urethra for males	van Belkum *et al.* (1995) Journal of Clinical Microbiology, 33(11), 2957-62
33	419bp	LCR	7405 (7191, 7192, 7193, 7231 not sequenced)	99.8	STD clinic; male and female patients from the Netherlands	1/53	cervical smears for females; swabs from urethra for males	van Belkum *et al.* (1995) Journal of Clinical Microbiology, 33(11), 2957-62
9	419bp	LCR	7311, 7312	99.5	STD clinic; male and female patients from the Netherlands	1/53	cervical smears for females; swabs from urethra for males	van Belkum *et al.* (1995) Journal of Clinical Microbiology, 33(11), 2957-62
7	419bp	LCR	7231, 7357, 7358	99.3	STD clinic; male and female patients from the Netherlands	1/53	cervical smears for females; swabs from urethra for males	van Belkum *et al.* (1995) Journal of Clinical Microbiology, 33(11), 2957-62

1	419bp	LCR	7429, 7430, 7431, 7433	99.0	STD clinic; male and female patients from the Netherlands	1/53	cervical smears for females; swabs from urethra for males	van Belkum *et al.* (1995) Journal of Clinical Microbiology, 33(11), 2957-62
10	419bp	LCR	7192, 7314, 7385, 7386	99.0	STD clinic; male and female patients from the Netherlands	1/53	cervical smears for females; swabs from urethra for males	van Belkum *et al.* (1995) Journal of Clinical Microbiology, 33(11), 2957-62
S83	1386bp Note: 1386bp region is from nts 4271 to 5656 of prototype; L2 region adjusted 1bp to align sequence	L2	5224	99.9	Patients From New Mexico and Oregon Note: 7 samples from U.K; 4 from Trinidad	1/41 (4/41 prototype)	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53; Wheeler *et al.* (1997) Journal of Clinical Microbiology, 35(1), 11-19
OR.5110	1386bp	L2	4736, 5224	99.8	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.4997	1386bp	L2	4936, 5224(T)	99.8	Patients From New Mexico and Oregon	2/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53

OR.6170	1386bp	L2	4408, 4936, 5224	99.8	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.6311	1386bp	L2	4309, 4936, 5224	99.8	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
114/B	1386bp	L2	4936, 5224, 5423	99.8	Patients From New Mexico and Oregon	1/41 (Keratinocyte HPV16 cell line)	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
114/K	1386bp	L2	4936, 5137, 5224(T), 5230	99.7	Patients From New Mexico and Oregon	1/41 (Keratinocyte HPV 16 cell line)	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
S29	1386bp	L2	4417, 4555(C), 4936, 5224	99.7	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
S27	1386bp	L2	4936, 5039, 5224(G), 5377	99.7	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.8329	1386bp	L2	4936, 4961, 5143, 5224(T)	99.7	Patients From New Mexico and Oregon	2/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53

OR.4724	1386bp	L2	4936, 5030, 5039, 5224	99.7	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.5428	1386bp	L2	4936, 5039, 5224, 5516	99.7	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
NM.T446	1386bp	L2	4612, 4936, 5039, 5224(C)	99.7	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
S93	1386bp	L2	4459, 4936, 5039, 5224, 5279	99.6	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.9237	1386bp	L2	4612, 4936, 5039, 5224(G), 5385	99.6	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
NM.T197	1386bp	L2	4612, 4936, 5039, 5224, 5234	99.6	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
S23	1386bp	L2	4723, 4936, 5043, 5224(T), 5377, 5516	99.6	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53

OR.0198	1386bp	L2	4510, 4612, 4936, 5039, 5224(G), 5234	99.6	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
NM.T455	1386bp	L2	4417, 4723, 4936, 5032, 5224, 5344	99.6	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.2087	1386bp	L2	4723, 4936, 5043, 5224, 5367, 5516	99.6	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.4716	1386bp	L2	4723, 4936, 5039, 5043, 5224, 5367, 5516	99.5	Patients From New Mexico and Oregon	2/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
0R.1905	1386bp	L2	4279, 4306, 4426, 4459, 4598, 4642, 4723, 4822, 4909, 4936, 5039, 5140, 5234, 5288, 5385, 5387, 5401, 5493, 5504, 5562	98.6	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.7587	1386bp	L2	4279, 4306, 4426, 4459, 4598, 4639, 4642, 4723, 4909, 4936, 5039, 5140, 5234, 5288, 5308, 5377, 5387, 5401, 5493, 5504, 5562	98.5	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53

OR.7632	1386bp	L2	4279, 4306, 4426, 4459, 4598, 4639, 4642, 4723, 4909, 4936, 5039, 5140, 5234, 5245, 5288, 5308, 5377, 5387, 5401, 5493, 5504, 5562	98.4	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.6106	1386bp	L2	4279, 4306, 4426, 4459, 4598, 4642, 4723, 4808, 4909, 4936, 5039, 5140, 5234, 5245, 5288, 5308, 5377, 5387, 5401, 5493, 5504, 5562	98.4	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.3473	1386bp	L2	4279, 4426(T), 4516, 4543, 4555, 4598, 4642, 4723, 4852, 4885, 4936, 5039, 5140, 5234, 5257, 5288(C), 5308, 5367, 5377, 5387, 5401, 5485, 5493, 5504, 5562	98.2	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.7145	1386bp	L2	4279, 4426(T), 4516, 4543, 4555, 4598, 4642, 4723, 4852, 4885, 4936, 5039, 5140, 5234, 5257, 5288(C), 5308, 5367, 5377, 5387, 5401, 5485, 5493, 5494, 5504, 5562	98.1	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53

OR.3759	1386bp	L2	4279, 4405, 4426(T), 4459, 4516, 4543, 4598, 4642, 4723, 4852, 4885, 4936, 5039, 5140, 5234, 5257, 5288(C), 5308, 5367, 5377, 5387, 5401, 5485, 5493, 5504, 5562	98.1	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.3136	1386bp	L2	4279, 4426(T), 4450, 4459, 4598, 4642, 4723, 4885, 4936, 4948, 4967, 5032, 5039, 5071, 5140, 5150, 5234, 5284, 5308, 5366, 5367, 5377, 5384, 5387, 5401, 5485, 5493, 5504, 5530, 5562	97.8	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
NM.T529	1386bp	L2	4279, 4426(T), 4450, 4459, 4555, 4597, 4598, 4642, 4723, 4885, 4936, 4942, 4948, 4967, 5032, 5039, 5140, 5234, 5284, 5293, 5308, 5366, 5367, 5377, 5384, 5387, 5401, 5473, 5485, 5493, 5504, 5562	97.7	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53

OR.4541	1386bp	L2	4279, 4426(T), 4450, 4459, 4597, 4598, 4642, 4723, 4885, 4936, 4942, 4948, 4967, 5032, 5039, 5140, 5229, 5234, 5284, 5293, 5308, 5366, 5367, 5377, 5384, 5387, 5401, 5473, 5485, 5493, 5504, 5562	97.7	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.8160	1386bp	L2	4279, 4426(T), 4450, 4459, 4477, 4597, 4598, 4642, 4723, 4885, 4936, 4942, 4948, 4967, 5032, 5039, 5140, 5234, 5284, 5293, 5308, 5366, 5367, 5377, 5384, 5387, 5401, 5473, 5485, 5493, 5504, 5562	97.7	Patients From New Mexico and Oregon	2/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
NM.4094	1386bp	L2	4279, 4426(T), 4450, 4459, 4597, 4598, 4642, 4723, 4885, 4936, 4942, 4948, 4967, 5032, 5039, 5140, 5229, 5234, 5284, 5293, 5308, 5366, 5367, 5377, 5384, 5387, 5401, 5473, 5485, 5493, 5498, 5504, 5562	97.6	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53

Bt12	399bp Note: 399bp region is from nts 4022 to 4420 of prototype; L2 region adjusted 1bp to align sequence	L2	(4227 not sequenced)	100	Patients from Barbados and Netherlands	2/19 (6/19 Prototype)	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62
Dt4	399bp	L2	(4227 and 4232 not sequenced)	100	Patients from Barbados and Netherlands	1/19	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62
Bt6	399bp	L2	4227	99.7	Patients from Barbados and Netherlands	7/19	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62
Dc141	399bp	L2	4112, 4182, 4227	99.2	Patients from Barbados and Netherlands	2/19	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62
Dt24	399bp	L2	4193, 4227, 4232	99.2	Patients from Barbados and Netherlands	1/19	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62

Bt23	222bp Note: 222bp region is from nts 6373 to 6594; L1 region adjusted 1bp to align sequence	L1	6432	99.5	Patients from Barbados and Netherlands	13/19 (4/19 Prototype)	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62
Bt20	222bp	L1	6432, 6444	99.1	Patients from Barbados and Netherlands	1/19	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62
Bt5	222bp	L1	6432, 6480	99.1	Patients from Barbados and Netherlands	1/19	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62
OR.5110	1484bp Note: 1484bp region is from 5664-7147 of prototype; L1 region adjusted 1bp to align sequence (6917 adjusted 4bp)	L1	6240	99.9	Patients From New Mexico and Oregon; Note: 7 samples from U.K; 4 from Trinidad	5/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53; Wheeler *et al.* (1997) Journal of Clinical Microbiology, 35(1), 11-19
OR.4997	1484bp	L1	6432, 6240	99.9	Patients From New Mexico and Oregon	5/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53

NM.T455	1484bp	L1	6111, 6240	99.9	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.6311	1484bp	L1	5960, 6240, 6432	99.8	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.2087	1484bp	L1	6240, 6432, 7058(A)	99.8	Patients From New Mexico and Oregon	3/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
NM.T446	1484bp	L1	6240, 6432, 6860	99.8	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
114/B	1484bp	L1	6216, 6240, 6432	99.8	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
S93	1484bp	L1	6240, 6389, 6432	99.8	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
S23	1484bp	L1	6163, 6240, 7058(A)	99.8	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53

S99	1484bp	L1	6111, 6240, 6432, 6860	99.7	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
T45	1484bp	L1	6240, 6389, 6432, 6454	99.7	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.7574	1484bp	L1	6179, 6240, 6432, 7058(A)	99.7	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
NM.T197	1484bp	L1	6207, 6240, 6432, 6860	99.7	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.9237	1484bp	L1	6240, 6432, 6860, 6963	99.7	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.0198	1484bp	L1	6240, 6432, 6860, 7076	99.7	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
T3	1484bp	L1	5877, 6178, 6240, 6304, 6575, 6913, 6973	99.5	Patients From New Mexico and Oregon	3/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53

OR.3136	1484bp	L1	5696, 5862, 5909, 6152, 6240, 6245, 6314, 6432, 6557, 6693, 6719, 6852, 6863, 6968, 6992, 7058	99.0	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.7587	1484bp	L1	5696, 5862, 5909, 6152, 6240, 6245, 6314, 6432, 6557, 6566, 6575, 6719, 6852, 6968, 6992, 7058	98.9	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
NM.4094	1484bp	L1	5696, 5862, 5909, 6152, 6163, 6240, 6245, 6432, 6557, 6693, 6719, 6852, 6863, 6968, 6992, 7058	98.9	Patients From New Mexico and Oregon	4/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.3759	1484bp	L1	5696, 5862, 5909, 6152, 6240, 6245, 6314, 6432, 6480, 6557, 6693, 6719, 6852, 6863, 6968, 6992, 7058	98.9	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.7632	1484bp	L1	5696, 5862, 5909, 6152, 6240, 6245, 6314, 6432, 6557, 6566, 6575, 6719, 6824, 6852, 6968, 6992, 7058	98.9	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
NM.T529	1484bp	L1	5696, 5862, 5909, 6152, 6163, 6240, 6245, 6432, 6539, 6557, 6693, 6719, 6852, 6863, 6968, 6992, 7058	98.9	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53

OR.3473	1484bp	L1	5696, 5862, 5909, 6152, 6163, 6240, 6245, 6314, 6432, 6480, 6557, 6693, 6719, 6852, 6863, 6968, 6992, 7058	98.8	Patients From New Mexico and Oregon	2/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.1905	1484bp	L1	5696, 5862, 5909, 5960, 6152, 6240, 6245, 6314, 6432, 6557, 6566, 6719, 6852, 6916, 6968, 6992, 7058, 7085	98.8	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.6106	1484bp	L1	5696, 5862, 5909, 5960, 6152, 6240, 6245, 6314, 6432, 6557, 6566, 6575, 6719, 6852, 6916, 6968, 6992, 7058, 7085	98.7	Patients From New Mexico and Oregon	1/41	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
IS.645	382bp Note: 382bp region is from nts 6616 to 6997 of prototype; L1 region adjusted 2bp to align sequence; insertion of 'CAT' at 6903 compared to prototype and thus a change at their 6904; 5bp adjustment at 6931	L1	6693	99.7	Patients from 22 countries worldwide	1/28 (6/28 prototype)	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72

IS.1032	382bp	L1	6824	99.7	Patients from 22 countries worldwide	2/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.845	382bp	L1	6719, 6852, 6968, 6992	99.0	Patients from 22 countries worldwide	3/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.393	382bp	L1	6719, 6852, 6926, 6968, 6992	98.7	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.347	382bp	L1	6719, 6852, 6866, 6968, 6992	98.7	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS. 21	382bp	L1	6693, 6852, 6863, 6968, 6992	98.7	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.812	382bp	L1	6693, 6719(C), 6852, 6863, 6968, 6992	98.4	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72

IS.846	382bp	L1	6693, 6719, 6852, 6863, 6968, 6992	98.4	Patients from 22 countries worldwide	5/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.170	382bp	L1	6693, 6719, 6852, 6863, '6904', 6968, 6992	98.2	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.808	382bp	L1	6693, 6719, 6852, 6863, 6956, 6968, 6992	98.2	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.177	382bp	L1	6693, 6719, 6824, 6852, 6863, 6968, 6992	98.2	Patients from 22 countries worldwide	2/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.545	382bp	L1	6693, 6719, 6801, 6852, 6863, 6968, 6992	98.2	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS. 53	382bp	L1	6693, 6719, 6852, 6860, 6863, 6968, 6992	98.2	Patients from 22 countries worldwide	2/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72

77	350bp Note: 350bp region is from nts 6268 to 6617 from prototype; L1 region adjusted 1bp to align sequence	L1	6314, 6432, 6480, 6557	98.9	HIV positive prostitutes from Zaire	4/4	cervicovaginal lavages	Icenogle *et al.* (1992) The Journal of Infectious Diseases, 166, 1210-6
1194	347bp Note: 347bp region is from nts 6656 to 7005 from prototype; L1 region adjusted 1bp to align sequence	L1	6693, 6719, 6852, 6863, 6968, 6992	98.3	HIV positive prostitutes from Zaire	2/3	cervicovaginal lavages	Icenogle *et al.* (1992) The Journal of Infectious Diseases, 166, 1210-6
84	347bp	L1	6693, 6719, 6852, 6863, 6961, 6968, 6992	98.0	HIV positive prostitutes from Zaire	1/3	cervicovaginal lavages	Icenogle *et al.* (1992) The Journal of Infectious Diseases, 166, 1210-6
Alaska - C32 (SiHa, Caski)	268bp Note: 268bp region is from nts 6346 to 6613 from prototype; L1 region adjusted 1bp to align sequence	L1	6432	99.6	Patients from locations worldwide	6/32 (1 Alaska, 4 Panama) prototype 14/32	cervical scrapes, cervical biopsies, cervicovaginal lavages, condylomas	Icenogle *et al.* (1991) Virology, 184, 101-7

Alabama-B24	268bp	L1	6432, 6530	99.3	Patients from locations worldwide	1/32	cervical scrapes, cervical biopsies, cervicovaginal lavages, condylomas	Icenogle *et al.* (1991) Virology, 184, 101-7
Panama-349	268bp	L1	6432, 6581	99.3	Patients from locations worldwide	2/32 (1 Panama, 1 Missouri)	cervical scrapes, cervical biopsies, cervicovaginal lavages, condylomas	Icenogle *et al.* (1991) Virology, 184, 101-7
Panama-200	268bp	L1	6432, 6557	99.3	Patients from locations worldwide	3/32 (2 Panama, 1 Georgia)	cervical scrapes, cervical biopsies, cervicovaginal lavages, condylomas	Icenogle *et al.* (1991) Virology, 184, 101-7
Alabama-B23	268bp	L1	6332, 6557, 6566	98.9	Patients from locations worldwide	1/32 Note: 5 other samples, same nucleotide changes mentioned amongst these 6 variants but sequences not given	cervical scrapes, cervical biopsies, cervicovaginal lavages, condylomas	Icenogle *et al.* (1991) Virology, 184, 101-7

OR.6170	456bp Note: 456bp region is from nts 104 to 559 of prototype	E6	109	99.8	Patients From New Mexico and Oregon	1/32 (3/32 prototype)	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53; Wheeler *et al.* (1997) Journal of Clinical Microbiology, 35(1), 11-19
OR.2087	456bp	E6	178	99.8	Patients From New Mexico and Oregon	4/32	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.6311	456bp	E6	256, 350	99.6	Patients From New Mexico and Oregon	1/32	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.8329	456bp	E6	109, 350	99.6	Patients From New Mexico and Oregon	2/32	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
NM.T197	456bp	E6	131, 350	99.6	Patients From New Mexico and Oregon	4/32	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.4997	456bp	E6	267, 269, 350	99.3	Patients From New Mexico and Oregon	1/32	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.3136	456bp	E6	145, 286, 289, 335, 350	98.9	Patients From New Mexico and Oregon	1/32	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53

OR.7587	456bp	E6	132, 143, 145, 286, 289, 335	98.7	Patients From New Mexico and Oregon	4/32	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
NM.T529	456bp	E6	145, 286, 289, 335, 350, 532	98.7	Patients From New Mexico and Oregon	5/32	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.5691	456bp	E6	145, 183, 286, 289, 335, 350, 532	98.5	Patients From New Mexico and Oregon	3/32	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
OR.3473	456bp	E6	109, 132(T), 143, 145, 286, 289, 335, 403	98.2	Patients From New Mexico and Oregon	3/32	cervical cell samples	Yamada *et al.* (1995) Journal of Virology, 69(12), 7743-53
IS.645	456bp Note: 456bp region is from nts 104 to 559 of prototype	E6	350	99.8	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.105	456bp	E6	176	99.8	Patients from 22 countries worldwide	2/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72

IS.925	456bp	E6	178	99.8	Patients from 22 countries worldwide	2/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.364	456bp	E6	188	99.8	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.463	456bp	E6	176, 350(A)	99.6	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.489	456bp	E6	176, 350	99.6	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS. 7	456bp	E6	188, 350	99.6	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS. 21	456bp	E6	145, 286, 289, 350, 532	98.9	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72

IS. 42	456bp	E6	145, 286, 289, 335, 350	98.9	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.808	456bp	E6	143, 145, 286, 289, 335	98.9	Patients from 22 countries worldwide	4/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.111	456bp	E6	145, 286, 289, 335, 350, 532	98.7	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.830	456bp	E6	132(C), 143, 145, 286, 289, 335	98.7	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.170	456bp	E6	143, 145, 286, 289, 335, 403	98.7	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.393	456bp	E6	132, 143, 145, 286, 289, 335	98.7	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72

IS.845	456bp	E6	131, 143, 145, 188, 286, 289, 335	98.5	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.811	456bp	E6	137, 143, 145, 285, 286, 289, 335	98.5	Patients from 22 countries worldwide	2/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.545	456bp	E6	145, 176, 286, 289, 335, 350, 532	98.5	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS. 53	456bp	E6	145, 183, 286, 289, 335, 350, 532	98.5	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.398	456bp	E6	131, 143, 145, 286, 289, 295, 335, 350	98.2	Patients from 22 countries worldwide	2/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
IS.812	456bp	E6	109, 132, 143, 145, 286, 289, 335, 403	98.8	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72

IS.347	456bp	E6	143, 145, 161, 255, 286, 289, 295, 335, 350	98.0	Patients from 22 countries worldwide	1/28	cervical cancer specimens	Yamada *et al.* (1997) Journal of Virology, 71(3), 2463-72
HHH66	167bp Note: 167bp region is from nucleotides 25 to 191 of prototype + amplified products were hybridized to E6 probes that target sequence variations at 109, 131, 132, 143, 145, 178, 183, 286, 289, 335, 350, 403 and 532	E6	350	NA	STD clinic patients and university students (Washington)	3/19 (4/19 prototype)	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802
HHH103	167bp + amplified products were hybridized to E6 probes that target sequence variations at 109, 131, 132, 143, 145, 178, 183, 286, 289, 335, 350, 403 and 532	E6	131	NA	STD clinic patients and university students (Washington)	1/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802

HHH245	167bp + amplified products were hybridized to E6 probes that target sequence variations at 109, 131, 132, 143, 145, 178, 183, 286, 289, 335, 350, 403 and 532	E6	42	NA	STD clinic patients and university students (Washington)	1/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802
HHH7	167bp + amplified products were hybridized to E6 probes that target sequence variations at 109, 131, 132, 143, 145, 178, 183, 286, 289, 335, 350, 403 and 532	E6	80	NA	STD clinic patients and university students (Washington)	2/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802
HHH81	167bp + amplified products were hybridized to E6 probes that target sequence variations at 109, 131, 132, 143, 145, 178, 183, 286, 289, 335, 350, 403 and 532	E6	178	NA	STD clinic patients and university students (Washington)	1/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802

HHH170	167bp + amplified products were hybridized to E6 probes that target sequence variations at 109, 131, 132, 143, 145, 178, 183, 286, 289, 335, 350, 403 and 532	E6	109	NA	STD clinic patients and university students (Washington)	1/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802
HHH104	167bp + amplified products were hybridized to E6 probes that target sequence variations at 109, 131, 132, 143, 145, 178, 183, 286, 289, 335, 350, 403 and 532	E6	109, 350	NA	STD clinic patients and university students (Washington)	1/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802
HHH228	167bp + amplified products were hybridized to E6 probes that target sequence variations at 109, 131, 132, 143, 145, 178, 183, 286, 289, 335, 350, 403 and 532	E6	131, 350	NA	STD clinic patients and university students (Washington)	1/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802

HHH208	167bp + amplified products were hybridized to E6 probes that target sequence variations at 109, 131, 132, 143, 145, 178, 183, 286, 289, 335, 350, 403 and 532	E6	145, 286, 289, 335, 350, 532	NA	STD clinic patients and university students (Washington)	2/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802
HHH5	167bp + amplified products were hybridized to E6 probes that target sequence variations at 109, 131, 132, 143, 145, 178, 183, 286, 289, 335, 350, 403 and 532	E6	31, 83, 132, 143, 145, 286, 289, 335	NA	STD clinic patients and university students (Washington)	1/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802
HHH198	167bp + amplified products were hybridized to E6 probes that target sequence variations at 109, 131, 132, 143, 145, 178, 183, 286, 289, 335, 350, 403 and 532	E6	31, 43, 109, 132(T), 143, 145, 286, 289, 335, 403	NA	STD clinic patients and university students (Washington)	1/19	cervical and vulvovaginal swabs	Xi *et al.* (1997) Journal of the National Cancer Institute, 89, 796-802
Specimen 37	303bp Note: 303bp region is from nts 118 to 420 of prototype	E6	176	99.7	Patients from Whittington Hospital, London, England	1/47 (21/47 prototype)	cervical scrapes	Londesborough *et al.* (1996) International Journal of Cancer, 69, 364-8

Specimen 40	303bp	E6	212	99.7	Patients from Whittington Hospital, London, England	1/47	cervical scrapes	Londesborough *et al.* (1996) International Journal of Cancer, 69, 364-8
Specimen 1	303bp	E6	350	99.7	Patients from Whittington Hospital, London, England	14/47	cervical scrapes	Londesborough *et al.* (1996) International Journal of Cancer, 69, 364-8
Specimen 13	303bp	E6	181, 350	99.3	Patients from Whittington Hospital, London, England	1/47	cervical scrapes	Londesborough *et al.* (1996) International Journal of Cancer, 69, 364-8
Specimen 29	303bp	E6	207, 350	99.3	Patients from Whittington Hospital, London, England	1/47	cervical scrapes	Londesborough *et al.* (1996) International Journal of Cancer, 69, 364-8
Specimen 33	303bp	E6	131, 350	99.3	Patients from Whittington Hospital, London, England	1/47	cervical scrapes	Londesborough *et al.* (1996) International Journal of Cancer, 69, 364-8
Specimen 34	303bp	E6	344, 350	99.3	Patients from Whittington Hospital, London, England	1/47	cervical scrapes	Londesborough *et al.* (1996) International Journal of Cancer, 69, 364-8
Specimen 38	303bp	E6	335, 350	99.3	Patients from Whittington Hospital, London, England	1/47	cervical scrapes	Londesborough *et al.* (1996) International Journal of Cancer, 69, 364-8

Specimen 41	303bp	E6	286, 289, 335	99.0	Patients from Whittington Hospital, London, England	2/47	cervical scrapes	Londesborough *et al.* (1996) International Journal of Cancer, 69, 364-8
Specimen 12	303bp	E6	145, 286, 289, 335, 350	98.3	Patients from Whittington Hospital, London, England	2/47	cervical scrapes	Londesborough *et al.* (1996) International Journal of Cancer, 69, 364-8
Specimen 31	303bp	E6	143, 145, 286, 289, 335, 350	98.0	Patients from Whittington Hospital, London, England	1/47	cervical scrapes	Londesborough *et al.* (1996) International Journal of Cancer, 69, 364-8
Seq1	524bp Note: 534bp region is from nts 52 to 575 of prototype	E6	256	99.8	patients from Sweden	1/42 (36/42 prototype; 22 CINIII, 14 carcinoma)	cervical carcinoma biopsies or punch biopsies of CIN III patients	Zehbe *et al.* (1998) Cancer Research, 58, 829-33
Seq3	524bp	E6	146	99.8	patients from Sweden	1/42 (CIN III)	cervical carcinoma biopsies or punch biopsies of CIN III patients	Zehbe *et al.* (1998) Cancer Research, 58, 829-33
Seq5	524bp	E6	139	99.8	patients from Sweden	1/42 (carcinoma)	cervical carcinoma biopsies or punch biopsies of CIN III patients	Zehbe *et al.* (1998) Cancer Research, 58, 829-33

Seq2	524bp	E6	286, 289, 532	99.4	patients from Sweden	2/42 (1 CIN III, 1 carcinoma)	cervical carcinoma biopsies or punch biopsies of CIN III patients	Zehbe *et al.* (1998) Cancer Research, 58, 829-33
Seq4	524bp	E6	109, 286, 289, 403	99.2	patients from Sweden	1/42 (1 CIN III)	cervical carcinoma biopsies or punch biopsies of CIN III patients	Zehbe *et al.* (1998) Cancer Research, 58, 829-33
Seq6	506bp Note: 506bp region is from nts 480 to 985 of prototype	E7	822	99.8	patients from Sweden	3/42 (2 CIN III, 1 carcinoma) (35/42 prototype; 20 CIN III, 15 carcinoma)	cervical carcinoma biopsies or punch biopsies of CIN III patients	Zehbe *et al.* (1998) Cancer Research, 58, 829-33
Seq7	506bp	E7	666	99.8	patients from Sweden	1/42 (1 CIN III)	cervical carcinoma biopsies or punch biopsies of CIN III patients	Zehbe *et al.* (1998) Cancer Research, 58, 829-33
Seq4	506bp	E7	789, 795	99.6	patients from Sweden	1/42 (1 CIN III)	cervical carcinoma biopsies or punch biopsies of CIN III patients	Zehbe *et al.* (1998) Cancer Research, 58, 829-33

Seq2	506bp	E7	732, 789, 795	99.4	patients from Sweden	2/42 (1 CIN III, 1 carcinoma)	cervical carcinoma biopsies or punch biopsies of CIN III patients	Zehbe *et al.* (1998) Cancer Research, 58, 829-33
Dt24	321bp Note: 321bp region is from nts 543 to 863 of prototype	E7	616	99.7	Patients from Barbados and Netherlands	1/19 (10/19 prototype)	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62
Bt7	321bp	E7	789, 795	99.4	Patients from Barbados and Netherlands	6/19	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62
Bt8	321bp	E7	732, 789, 795	99.1	Patients from Barbados and Netherlands	2/19	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62
A27	~240-294bp Note: region is approximately from nts 562 to 855 of prototype	E7	790	NA	Patients from Germany or Tanzania	1/32 (1CC) (13/32 prototype)	genital warts (GW); cervical carcinomas (CC); penile carcinoma (PC); vaginal carcinoma (VC)	Eschle *et al.* (1992) Journal of General Virology, 73, 1829-32

A36	~240-294bp	E7	789, 795	NA	Patients from Germany or Tanzania	10/32 (9CC, 1VC)	genital warts (GW); cervical carcinomas (CC); penile carcinoma (PC); vaginal carcinoma (VC)	Eschle *et al.* (1992) Journal of General Virology, 73, 1829-32
A6	~240-294bp	E7	647, 789, 795	NA	Patients from Germany or Tanzania	8/32 (8CC)	genital warts (GW); cervical carcinomas (CC); penile carcinoma (PC); vaginal carcinoma (VC)	Eschle *et al.* (1992) Journal of General Virology, 73, 1829-32
84	945bp Note: 945bp region is from nts26-970 of prototype	E7	647, 789, 795	99.7	HIV positive prostitutes from Zaire	2/2	cervicovaginal lavages	Icenogle *et al.* (1992) The Journal of Infectious Diseases, 166, 1210-6
Panama-F30	220bp Note: 220bp region is from nts 582 to 801 of prototype	E7	666	99.5	Patients from locations worldwide	1/32 (14/32 prototype)	cervical scrapes, cervical biopsies, cervicovaginal lavages, condylomas	Icenogle *et al.* (1991) Virology, 184, 101-7
SiHa	220bp	E7	645	99.5	Patients from locations worldwide	1/32	cervical scrapes, cervical biopsies, cervicovaginal lavages, condylomas	Icenogle *et al.* (1991) Virology, 184, 101-7

Panama-C20	220bp	E7	678	99.5	Patients from locations worldwide	1/32	cervical scrapes, cervical biopsies, cervicovaginal lavages, condylomas	Icenogle *et al.* (1991) Virology, 184, 101-7
Missouri-B11	220bp	E7	701	99.5	Patients from locations worldwide	1/32	cervical scrapes, cervical biopsies, cervicovaginal lavages, condylomas	Icenogle *et al.* (1991) Virology, 184, 101-7
Panama-200	220bp	E7	789, 795	99.1	Patients from locations worldwide	1/32	cervical scrapes, cervical biopsies, cervicovaginal lavages, condylomas	Icenogle *et al.* (1991) Virology, 184, 101-7
Panama-C10	220bp	E7	647, 789, 795	98.6	Patients from locations worldwide	1/32	cervical scrapes, cervical biopsies, cervicovaginal lavages, condylomas	Icenogle *et al.* (1991) Virology, 184, 101-7

Panama-B20	220bp	E7	732, 789, 795	98.6	Patients from locations worldwide	4/32 (2 Panama, 1 Georgia, 1 Alabama) Note: 8 other samples, same nucleotide changes mentioned amongst these 6 variants but sequences not given	cervical scrapes, cervical biopsies, cervicovaginal lavages, condylomas	Icenogle *et al.* (1991) Virology, 184, 101-7
Bt6	247bp Note: 247bp region is from nts 3835 to 4081 of prototype; E5 region adjusted 1bp or 2bp (3884 on) to align sequence	E5	3866	99.6	Patients from Barbados and Netherlands	1/19 (3/19 prototype)	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62
Bt23	247bp	E5	3977, 4040	99.2	Patients from Barbados and Netherlands	6/19	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62
Dc269	247bp	E5	3882, 3977, 4040	98.8	Patients from Barbados and Netherlands	1/19	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62

Bt8	247bp	E5	3857, 3977, 3989, 4040	98.4	Patients from Barbados and Netherlands	2/19	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62
Bt7	247bp	E5	3857, 3977, 3989, 4040, 4057	98.0	Patients from Barbados and Netherlands	1/19	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62
Bt9	247bp	E5	3857, 3867, 3977, 3989, 4040	98.0	Patients from Barbados and Netherlands	3/19	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62
Bt22	247bp	E5	3857, 3867, 3977, 3989, 4040 (3866 and 4057 not sequenced)	98.0	Patients from Barbados and Netherlands	1/19	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62
Bt10	247bp	E5	3857, 3867, 3977, 3989, 4040, 4048	97.6	Patients from Barbados and Netherlands	1/19	cervical lesions and cervical cancer specimens	Smits *et al.* (1994) Journal of General Virology, 75, 2457-62

OR. 5110	252bp Note: 252bp region is from nts 3848 to 4099 of prototype; E5 region adjusted 1bp and 2bp (3967 on) to align sequence	E5	3866	99.6	Patients worldwide + Oregon	1/45 (3/45 prototype)	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
OR.6170	252bp	E5	3859	99.6	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
IS.244	252bp	E5	4040	99.6	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
OR.4997	252bp	E5	3977(G), 4040	99.2	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
OR.4724	252bp	E5	3977, 4040	99.2	Patients worldwide + Oregon	9/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600

OR.6311	252bp	E5	3977, 3993, 4040	98.8	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
OR.8329	252bp	E5	3977, 4040, 4087	98.8	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
OR.2087	252bp	E5	3977, 4040, 4075	98.8	Patients worldwide + Oregon	3/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
IS.825	252bp	E5	3857, 3977, 3989, 4087	98.4	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
IS.811	252bp	E5	3857, 3977, 3989, 4040(C), 4087	98.0	Patients worldwide + Oregon	2/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
OR.3759	252bp	E5	3857, 3977, 3989, 4040, 4087	98.0	Patients worldwide + Oregon	2/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600

OR.7145	252bp	E5	3857, 3977, 3989(G), 4040, 4087	98.0	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
OR.7587	252bp	E5	3857, 3867, 3977, 3989, 4040(T), 4087	97.6	Patients worldwide + Oregon	5/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
OR.1905	252bp	E5	3857, 3867, 3977, 3989, 4040, 4087	97.6	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
IS.347	252bp	E5	3857, 3867(C), 3977, 3989(G), 4040, 4087(G)	97.6	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
IS.808	252bp	E5	3857, 3977, 3989, 4028, 4040, 4087	97.6	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
OR.3136	252bp	E5	3857, 3977, 3989, 4015, 4040, 4087	97.6	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600

OR.4541	252bp	E5	3857, 3977, 3989(G), 4015, 4040, 4087	97.6	Patients worldwide + Oregon	6/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
IS. 21	252bp	E5	3857, 3977, 3988, 3989, 4015, 4040,	97.6	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
OR.5691	252bp	E5	3857, 3965, 3977, 3989, 4015, 4040,	97.6	Patients worldwide + Oregon	3/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
Sb-2	252bp Note: 252bp region is from nts 3848 to 4099 of prototype; E5 region adjusted 1bp and 2bp (3967 on) to align sequence	E5	3977, 4033, 4040	98.8	Patients from Singapore, Brazil, Tanzania and Germany	1/22 (3/22 prototype)	cervical smears, biopsy specimens from cervical carcinoma and biopsy specimens from CIN I, II, III	Chan *et al.* (1992) Journal of Virology, 66(4), 2057-66
Caski	252bp	E5	3971, 3977, 4040	98.8	Patients from Singapore, Brazil, Tanzania and Germany	1/22	cervical smears, biopsy specimens from cervical carcinoma and biopsy specimens from CIN I, II, III	Chan *et al.* (1992) Journal of Virology, 66(4), 2057-66

Sb-7	252bp	E5	3977, 4040, 4075	98.8	Patients from Singapore, Brazil, Tanzania and Germany	2/22	cervical smears, biopsy specimens from cervical carcinoma and biopsy specimens from CIN I, II, III	Chan *et al.* (1992) Journal of Virology, 66(4), 2057-66
Sb-5	252bp	E5	3871, 3968, 3977, 4040	98.4	Patients from Singapore, Brazil, Tanzania and Germany	1/22	cervical smears, biopsy specimens from cervical carcinoma and biopsy specimens from CIN I, II, III	Chan *et al.* (1992) Journal of Virology, 66(4), 2057-66
SiHa	252bp	E5	3902, 3977, 3989, 4040	98.4	Patients from Singapore, Brazil, Tanzania and Germany	1/22	cervical smears, biopsy specimens from cervical carcinoma and biopsy specimens from CIN I, II, III	Chan *et al.* (1992) Journal of Virology, 66(4), 2057-66
Sb-17	252bp	E5	3977, 4040, 4044, 4075	98.4	Patients from Singapore, Brazil, Tanzania and Germany	1/22	cervical smears, biopsy specimens from cervical carcinoma and biopsy specimens from CIN I, II, III	Chan *et al.* (1992) Journal of Virology, 66(4), 2057-66

Sb-13	252bp	E5	3977, 3980, 4038, 4040, 4075	98.0	Patients from Singapore, Brazil, Tanzania and Germany	1/22	cervical smears, biopsy specimens from cervical carcinoma and biopsy specimens from CIN I, II, III	Chan *et al.* (1992) Journal of Virology, 66(4), 2057-66
Sb-10	252bp	E5	3873, 3932, 3977, 4040, 4075	98.0	Patients from Singapore, Brazil, Tanzania and Germany	1/22	cervical smears, biopsy specimens from cervical carcinoma and biopsy specimens from CIN I, II, III	Chan *et al.* (1992) Journal of Virology, 66(4), 2057-66
Sb-21a	252bp	E5	3857, 3977, 3989, 4040, 4057, 4087	97.6	Patients from Singapore, Brazil, Tanzania and Germany	4/22	cervical smears, biopsy specimens from cervical carcinoma and biopsy specimens from CIN I, II, III	Chan *et al.* (1992) Journal of Virology, 66(4), 2057-66
Sb-16	252bp	E5	3857, 3977, 3989, 4015, 4040, 4087	97.6	Patients from Singapore, Brazil, Tanzania and Germany	3/22	cervical smears, biopsy specimens from cervical carcinoma and biopsy specimens from CIN I, II, III	Chan *et al.* (1992) Journal of Virology, 66(4), 2057-66

Tb-4	252bp	E5	3857, 3867, 3977, 3989(T), 4040(T), 4087	97.6	Patients from Singapore, Brazil, Tanzania and Germany	1/22	cervical smears, biopsy specimens from cervical carcinoma and biopsy specimens from CIN I, II, III	Chan *et al.* (1992) Journal of Virology, 66(4), 2057-66
Tb-16	252bp	E5	3857, 3866, 3867, 3977, 3989(T), 4040(T), 4087	97.2	Patients from Singapore, Brazil, Tanzania and Germany	1/22	cervical smears, biopsy specimens from cervical carcinoma and biopsy specimens from CIN I, II, III	Chan *et al.* (1992) Journal of Virology, 66(4), 2057-66
Tb-13	252bp	E5	3857, 3867, 3977(G), 3986, 3989(T), 4011, 4040(T), 4087	96.8	Patients from Singapore, Brazil, Tanzania and Germany	1/22	cervical smears, biopsy specimens from cervical carcinoma and biopsy specimens from CIN I, II, III	Chan *et al.* (1992) Journal of Virology, 66(4), 2057-66
OR.6331	249bp Note: 249bp region is from nts 3337 to 3570 of prototype; E2 region adjusted 1bp to align sequence	E2	3409	99.6	Patients worldwide + Oregon	8/45 (3/45 prototype)	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600

OR.4724	249bp	E2	3383, 3409	99.2	Patients worldwide + Oregon	3/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
OR.4997	249bp	E2	3409, 3415	99.2	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
IS. 30	249bp	E2	3373, 3409	99.2	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
OR.2087	249bp	E2	3383, 3409, 3448, 3523	98.4	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
OR.7574	249bp	E2	3383, 3409, 3448, 3523, 3532	97.9	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
IS.398	249bp	E2	3373, 3409, 3448, 3515, 3565	97.9	Patients worldwide + Oregon	3/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600

IS.393	249bp	E2	3373, 3409, 3424(T), 3448, 3515, 3565	97.6	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
IS. 21	249bp	E2	3373, 3409, 3448, 3515, 3516, 3537 (3566 indistiguishable)	97.6	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
OR.5428	249bp	E2	3383, 3409, 3448, 3496, 3516, 3523, 3532	97.2	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
OR.1905	249bp	E2	3361, 3373, 3409, 3424, 3448, 3515, 3565	97.2	Patients worldwide + Oregon	3/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
IS.812	249bp	E2	3373, 3409, 3448, 3515, 3516, 3537, 3565	97.2	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
OR.7587	249bp	E2	3361, 3373, 3409, 3424, 3448, 3462, 3515, 3565	96.8	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600

IS.808	249bp	E2	3373, 3409, 3430, 3448, 3515, 3516, 3537, 3565	96.8	Patients worldwide + Oregon	4/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
OR.3759	249bp	E2	3361, 3373, 3409, 3430, 3448, 3515, 3516, 3565	96.8	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
OR.7908	249bp	E2	3373, 3386, 3409, 3448, 3515, 3516, 3537, 3565	96.8	Patients worldwide + Oregon	4/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
OR.8392	249bp	E2	3373, 3409, 3415, 3448, 3515, 3516, 3537, 3565	96.8	Patients worldwide + Oregon	3/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
OR.3473	249bp	E2	3361, 3373, 3409, 3430, 3448, 3515, 3516, 3537, 3565	96.4	Patients worldwide + Oregon	2/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600
OR.4541	249bp	E2	3373, 3386, 3409, 3448, 3462, 3515, 3516, 3537, 3565	96.4	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600

OR.8160	249bp	E2	3373, 3386, 3409, 3448, 3515, 3516, 3523, 3532, 3537, 3565	96.0	Patients worldwide + Oregon	1/45	OR: cervicovaginal lavage samples & IS: cervical cancer specimens	Eriksson *et al.* (1999) Journal of General Virology, 80, 595-600

TABLE 5: HPV 16 FREQUENCY OF LONG CODING REGION (LCR) STRAINS
(ONLY NUCLEOTIDES 7477 TO 7840 OF PROTOTYPE INCLUDED)

STRAIN NAME	NUMBER OF STRAINS	FREQUENCY	STRAIN NAME	NUMBER OF STRAINS	FREQUENCY	STRAIN NAME	NUMBER OF STRAINS	FREQUENCY
PROTOTYPE	80	0.1909	HDS106a	2	0.0048	B-19	1	0.0024
S-2	136	0.3246	HHH101	2	0.0048	J-1	1	0.0024
IND-8	28	0.0668	HHH7	2	0.0048	T-S5b	1	0.0024
J-3	17	0.0405	HDS84b	1	0.0024	T-S5b	1	0.0024
S-H2a	16	0.0382	B-24	1	0.0024	T-S5c	1	0.0024
T-8	15	0.0358	B-3	1	0.0024	T-S3	1	0.0024
T-1	13	0.0310	G-13	1	0.0024	T-S3a	1	0.0024
S-7	9	0.0215	G-10	1	0.0024	T-S3b	1	0.0024
T-4	9	0.0215	IND-4	1	0.0024	T-S3c	1	0.0024
B-14	5	0.0119	IND-7	1	0.0024	B-8a	1	0.0024
S-21a	5	0.0119	AP-4	1	0.0024	T-15	1	0.0024
S-13	3	0.0072	SA-9	1	0.0024	IS.846	1	0.0024
AN-12	3	0.0072	SL-6a	1	0.0024	1S. 42	1	0.0024
T-16	3	0.0072	Ss-1a	1	0.0024	IS.105	1	0.0024

B-11	3	0.0072	S-14	1	0.0024	IS.1032	1	0.0024
S-5	2	0.0048	J-5	1	0.0024	IS.393	1	0.0024
IND-5	2	0.0048	S-21	1	0.0024	IS.838	1	0.0024
S-H17	2	0.0048	T-S6a	1	0.0024	OR.3136	1	0.0024
G-21a	2	0.0048	T-S6b	1	0.0024	OR.4531	1	0.0024
S-23	2	0.0048	AN-10	1	0.0024	OR.8392	1	0.0024
SN-4	2	0.0048	P-1	1	0.0024	HHH165	1	0.0024
T-S5a	2	0.0048	B-1	1	0.0024	HHH260	1	0.0024
IS.811	2	0.0048	T-S5	1	0.0024	HHH105	1	0.0024
IS.177	2	0.0048	B-19	1	0.0024	HHH198	1	0.0024
IS.170	2	0.0048	CO	1	0.0024	HHH208	1	0.0024
			C3	1	0.0024			
TOTAL OF 3 COLUMNS	419	1.00						

TABLE 6: HPV 18 GENOTYPE, VARIANTS AND SUBTYPES

STRAIN NAME	LENGTH	SPECIFIC GENE(S)	NUCLEOTIDE POSITION CHANGES	% HOMOLOGY TO PROTOTYPE	PATIENT POPULATION	# PATIENTS STRAINS FOUND	SOURCE	REFERENCE
HPV 18, prototype	7857bp	ALL Note: 321bp region is from nucleotides 7485-7805	NA	NA	NA	NA	cervical carcinoma	Cole *et al.* (1987) Journal of Molecular Biology, 193(4), 599-608
J18-1	321bp	LCR (long control region)	7592	99.7	Patients from 8 areas worldwide	10/54 (3 Japan, 1 Scotland, 6 Singapore) 7/54 prototype (3 Brazil, 3 Singapore, 1 Greece)	cervical carcinomas or precursor lesions	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31
AM18-1	321bp	LCR	7528, 7592	99.4	Patients from 8 areas worldwide	3/54 (2 Amazon, 1 Brazil)	cervical carcinomas or precursor lesions	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31

T18-9	321bp	LCR	7529, 7567, 7592, 7670	98.8	Patients from 8 areas worldwide	2/54 (1 Brazil, 1 Tanzania)	cervical carcinomas or precursor lesions	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31
G18-2	321bp	LCR	7529, 7567, 7592, 7670, 7681	98.4	Patients from 8 areas worldwide	1/54 (1 German)	cervical carcinomas or precursor lesions	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31
G18-1 (Hela)	321bp	LCR	7486, 7529, 7567, 7592, 7670	98.4	Patients from 8 areas worldwide	6/54 (3 Scotland, 1 Singapore, 1 Greece, 1 German)	cervical carcinomas or precursor lesions	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31
SC18-3	321bp	LCR	7527, 7529, 7567, 7592, 7670	98.4	Patients from 8 areas worldwide	2/54 (2 Scotland)	cervical carcinomas or precursor lesions	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31
H18-4	321bp	LCR	7486, 7563, 7567, 7592, 7670	98.4	Patients from 8 areas worldwide	1/54 (1 Greece)	cervical carcinomas or precursor lesions	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31

C4-1	321bp	LCR	7529, 7567, 7592, 7670, 7717	98.4	NA	NA	cell line	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31
B18-2	321bp	LCR	7486, 7529, 7563, 7567, 7592, 7670	98.1	Patients from 8 areas worldwide	1/54 (1 Brazil)	cervical carcinomas or precursor lesions	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31
SC18-4a	321bp	LCR	7507, 7529, 7564, 7567, 7592, 7670	98.1	Patients from 8 areas worldwide	2/54 (2 Scotland)	cervical carcinomas or precursor lesions	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31
SC18-2	321bp	LCR	7486, 7529, 7563, 7567, 7592, 7670, 7717(G)	97.8	Patients from 8 areas worldwide	1/54 (1 Scotland)	cervical carcinomas or precursor lesions	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31
SC18-4b	321bp	LCR	7486, 7529, 7536, 7563, 7567, 7592, 7670	97.8	Patients from 8 areas worldwide	1/54 (1 Scotland)	cervical carcinomas or precursor lesions	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31

T18-7	321bp	LCR	7529, 7530(A), 7563, 7567, 7592, 7670, 7717, 7726, 7730	97.2	Patients from 8 areas worldwide	1/54 (1 Tanzania)	cervical carcinomas or precursor lesions	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31
T18-10	321bp	LCR	7529, 7530, 7563, 7567, 7592, 7643, 7651, 7658, 7670, 7704, 7726, 7730	96.3	Patients from 8 areas worldwide	1/54 (1 Tanzania)	cervical carcinomas or precursor lesions	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31
T18-12	321bp	LCR	7529, 7530, 7563, 7567, 7592, 7643, 7651, 7658, 7670, 7704, 7717(G), 7726, 7730	96.0	Patients from 8 areas worldwide	1/54 (1 Tanzania)	cervical carcinomas or precursor lesions	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31
T18-3	321bp	LCR	7507, 7512, 7529, 7530, 7551, 7563, 7567, 7592, 7651, 7670, 7704, 7726, 7730	96.0	Patients from 8 areas worldwide	1/54 (1 Tanzania)	cervical carcinomas or precursor lesions	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31
T18-8	321bp	LCR	7496, 7507, 7529, 7530, 7563, 7567, 7592, 7643, 7651, 7658, 7670, 7704, 7726, 7730	95.6	Patients from 8 areas worldwide	7/54 (4 Tanzania, 2 Greece, 1 Scotland)	cervical carcinomas or precursor lesions	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31

T18-5	321bp	LCR	7507, 7512, 7529, 7530, 7563, 7567, 7592, 7643, 7651, 7658(T), 7670, 7704, 7726, 7730	95.6	Patients from 8 areas worldwide	1/54 (1 Tanzania)	cervical carcinomas or precursor lesions	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31
T18-4	321bp	LCR	7507, 7512, 7529, 7530, 7563, 7567, 7592, 7643, 7651, 7658, 7670, 7704, 7726, 7730, 7783	95.3	Patients from 8 areas worldwide	1/54 (1 Tanzania)	cervical carcinomas or precursor lesions	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31
T18-16	321bp	LCR	7507, 7512, 7529, 7530, 7549, 7563, 7567, 7592, 7643, 7651, 7658, 7670, 7704, 7726, 7730	95.3	Patients from 8 areas worldwide	1/54 (1 Tanzania)	cervical carcinomas or precursor lesions	Ong *et al.* (1993). Journal of Virology, 67(11), 6424-31
T18-17	321bp	LCR	7507, 7512, 7529, 7530, 7563, 7567, 7592, 7643, 7651, 7658, 7670, 7704, 7717(G), 7726, 7730	95.3	Patients from 8 areas worldwide	1/54 (1 Tanzania)	cervical carcinomas or precursor lesions	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31

T18-18a	321bp	LCR	7507, 7512, 7529, 7530, 7549(A), 7563, 7567, 7592, 7643, 7651, 7658, 7670, 7704, 7726, 7730	95.3	Patients from 8 areas worldwide	1/54 (1 Tanzania)	cervical carcinomas or precursor lesions	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31
T18-18b	321bp	LCR	7507, 7512, 7529, 7530, 7549(A), 7563, 7567, 7592, 7643, 7651, 7658, 7670, 7704, 7717(G), 7726, 7730	95.0	Patients from 8 areas worldwide	1/54 (1 Tanzania)	cervical carcinomas or precursor lesions	Ong *et al.* (1993) Journal of Virology, 67(11), 6424-31
NY18-1	321bp	LCR	7592, 7717	99.4	Patients from the United States	unknown (New York Variant)	unknown	Ong *et al.* (1996) unpublished
NY18-2	321bp	LCR	7512, 7529, 7563, 7567, 7592, 7670	98.1	Patients from the United States	unknown (New York Variant)	unknown	Ong *et al.* (1996) unpublished
NY18-6	321bp	LCR	7486, 7529, 7567, 7592, 7670, 7717	98.1	Patients from the United States	unknown (New York Variant)	unknown	Ong *et al.* (1996) unpublished
NY18-11	321bp	LCR	7486, 7529, 7567, 7592, 7670, 7704	98.1	Patients from the United States	unknown (New York Variant)	unknown	Ong *et al.* (1996) unpublished
A18-1	321bp	LCR	7486, 7529, 7536, 7567, 7592, 7670	98.1	Patients from the United States	unknown (American Indian Variant)	unknown	Ong *et al.* (1996) unpublished

A18-2	321bp	LCR	7486, 7529, 7563, 7567, 7592, 7670	98.1	Patients from the United States	unknown (American Indian Variant)	unknown	Ong *et al.* (1996) unpublished
C18-7	321bp	LCR	7529, 7563, 7567, 7592, 7670, 7717	98.1	Patients from the United States	unknown (North Carolina Variant)	unknown	Ong *et al.* (1996) unpublished
C18-5	321bp	LCR	7528(G), 7529, 7563, 7567, 7592, 7670, 7717	97.8	Patients from the United States	unknown (North Carolina Variant)	unknown	Ong *et al.* (1996) unpublished
IN18-1	321bp	LCR	7486, 7529, 7567, 7592, 7670, 7726, 7730	97.8	Patients from the United States	unknown (Sub-Continental Indian Variant)	unknown	Ong *et al.* (1996) unpublished
NY18-13	321bp	LCR	7486, 7528(G), 7529, 7564, 7567, 7592, 7670, 7704	97.5	Patients from the United States	unknown (New York Variant)	unknown	Ong *et al.* (1996) unpublished
T18-8N	321bp	LCR	7496, 7507, 7530, 7563, 7567, 7592, 7643, 7651, 7658, 7670, 7704, 7726, 7730	96.0	Patients from Mexico City	4/24 (16/24 prototype)	cervical carcinoma	Lizano *et al.* (1997) Journal of the National Cancer Institute, 89(16), 1227-31
T18-9	321bp	LCR	7529, 7567, 7592, 7670	98.8	Patients from Mexico City	4/24	cervical carcinoma	Lizano *et al.* (1997) Journal of the National Cancer Institute, 89(16), 1227-31

IS002	455bp	L1 Note: 455bp region is from nucleotides 6558-7012 of prototype	6625, 6842	99.6	Patients from 22 countries worldwide and New Mexico	33/44 2 Algeria, 1 Tanzania, 2 Thailand, 1 Philippines, 8 Indonesia, 3 Canada, 2 Argentina, 2 Brazil, 1 Bolivia, 1 Cuba, 1 Chile, 2 Columbia, 2 Panama, 2 Paraguay, 1 Poland, 2 Spain	cervical cancer specimen	Stewart *et al.* (1996) Journal of Virology, 70(5), 3127-36
IS326	455bp	L1	6625, 6677, 6697, 6842	99.1	Patients from 22 countries worldwide and New Mexico	1/44 Chile	cervical cancer specimen	Stewart *et al.* (1996) Journal of Virology, 70(5), 3127-36
IS664	455bp	L1	6625, 6842, 6877, 6943	99.1	Patients from 22 countries worldwide and New Mexico	1/44 Poland	cervical cancer specimen	Stewart *et al.* (1996) Journal of Virology, 70(5), 3127-36
IS168	455bp	L1	6579, 6581, 6625, 6626, 6719, 6749, 6842, 6917	98.2	Patients from 22 countries worldwide and New Mexico	4/44 Benin, Cuba, Uganda, Tanzania	cervical cancer specimen	Stewart *et al.* (1996) Journal of Virology, 70(5), 3127-36
IS768	455bp	L1	6579, 6581, 6625, 6626, 6719, 6749, 6842, 6917, 6943	98.0	Patients from 22 countries worldwide and New Mexico	1/44 Uganda	cervical cancer specimen	Stewart *et al.* (1996) Journal of Virology, 70(5), 3127-36
IS172	455bp	L1	6579, 6581, 6625, 6626, 6719, 6749, 6842, 6917, 6986	98.0	Patients from 22 countries worldwide and New Mexico	4/44 Benin, 2 Mali, Guinea	cervical cancer specimen	Stewart *et al.* (1996) Journal of Virology, 70(5), 3127-36

IS168 (T18-8N)	455bp	L1	6579, 6581, 6625, 6626, 6719, 6749, 6842, 6917	98.2	Patients from Mexico City	4/24 (16/24 prototype)	cervical carcinoma	Lizano *et al.* (1997) Journal of the National Cancer Institute, 89(16), 1227-31
IS002 (T18-9)	455bp	L1	6625, 6842	99.6	Patients from Mexico City	4/24	cervical carcinoma	Lizano *et al.* (1997) Journal of the National Cancer Institute, 89(16), 1227-31
2	154bp Note: 154bp region is from nucleotides 3479-3632 of prototype	E2	3630	99.4	unknown	3/15 (2/15 prototype)	cervical biopsy specimens	Hecht *et al.* (1995) International Journal of Cancer, 60, 369-76
16	154bp	E2	3525	99.4	unknown	1/15	cervical biopsy specimens	Hecht *et al.* (1995) International Journal of Cancer, 60, 369-76
3	154bp	E2	3492, 3630	98.7	unknown	1/15	cervical biopsy specimens	Hecht *et al.* (1995) International Journal of Cancer, 60, 369-76
14	154bp	E2	3482, 3630	98.7	unknown	2/15	cervical biopsy specimens	Hecht *et al.* (1995) International Journal of Cancer, 60, 369-76

4	154bp	E2	3534, 3558, 3578, 3586, 3593, 3630, 3632	95.5	unknown	3/15	cervical biopsy specimens	Hecht *et al.* (1995) International Journal of Cancer, 60, 369-76
27	154bp	E2	3534, 3558, 3586, 3593, 3617, 3630, 3632	95.5	unknown	1/15	cervical biopsy specimens	Hecht *et al.* (1995) International Journal of Cancer, 60, 369-76
8	154bp	E2	3513, 3534, 3558, 3578, 3586, 3593, 3630, 3632	94.8	unknown	1/15	cervical biopsy specimens	Hecht *et al.* (1995) International Journal of Cancer, 60, 369-76
23	154bp	E2	3534, 3558, 3563, 3578, 3586, 3593, 3630, 3632	94.8	unknown	1/15	cervical biopsy specimens	Hecht *et al.* (1995) International Journal of Cancer, 60, 369-76
C41	263bp Note: 263bp region is from nts. 629-891 of prototype	E7	751	99.6	NA	NA	Cell line	ter Meulen *et al.* (1993) International Journal of Cancer, 53, 257-9
SW756	263bp	E7	640, 865	99.2	NA	NA	Cell line	ter Meulen *et al.* (1993) International Journal of Cancer, 53, 257-9

HELA	263bp	E7	751, 806	99.2	NA	NA	Cell line	ter Meulen *et al.* (1993) International Journal of Cancer, 53, 257-9
B6	~260bp Note: regions differ by few nucleotides (nts. ~631-890 of prototype)	E7	640	99.7	Tanzanian and German patients	1/26 (Tanzania) (3/26 prototype)	cervical carcinoma	ter Meulen *et al.* (1993) International Journal of Cancer, 53, 257-9
B17	~260bp	E7	770	99.7	Tanzanian and German patients	3/26 (All Tanzania)	1 cervical carcinoma and 2 cervical scrapes	ter Meulen *et al.* (1993) International Journal of Cancer, 53, 257-9
101	~260bp	E7	751	99.7	Tanzanian and German patients	3/26 (All German)	cervical carcinomas	ter Meulen *et al.* (1993) International Journal of Cancer, 53, 257-9
A5	~260bp	E7	640, 864	99.3	Tanzanian and German patients	16/26 (All Tanzania)	11 cervical carcinomas and 5 cervical scrapes	ter Meulen *et al.* (1993) International Journal of Cancer, 53, 257-9
HPV 18 E6, E7, E1	1750bp Note: 912bp region is from nucleotides 94-1005 of prototype	E6/7	287	99.9	NA	NA	cervical carcinoma cells	Seedorf *et al.* (1987) EMBO, 6(1), 139-44

HPV 18 variant	3135bp	E6/7	287, 485, 549, 751, 806	99.5	NA	NA	Hela cell line	Inagaki *et al.* (1988) Journal of Virology, 62(5), 1640-6
HPV 18 E6, E7	912bp	E6/7	104, 169, 287, 549, 751, 806	99.3	NA	NA	Hela cell line	Schneider-Gaedicke *et al.* (1986) EMBO, 5, 2285-92
HPV 18 E6, E7	5210bp	E6/7	104, 287, 485, 549, 751, 806	99.3	NA	NA	Hela cell line	Meissner *et al.* unpublished
HPV18 E6	1188bp	E6	287, 725, 728, 731, 926; insertion at 651, 921-7; deletions at 773, 784, 884, 897	98.1	NA	NA	cevical carcinoma biopsy	Matlashewski *et al.* (1986) Journal of General Virology, 67(Pt 9), 1909-16
HPV 18 E6, E7	1300bp	E6/7	104, 485, 549, 751, 806; deletions 182, 236-417; many changes between 930-1005	75.9	NA	NA	Hela cell line	Inagaki *et al.* (1988) Journal of Virology, 62(5), 1640-6

TABLE 7: HPV 18 FREQUENCY OF L1 REGION STRAINS
(ONLY NUCLEOTIDES 6558 TO 7012 OF PROTOTYPE INCLUDED)

STRAIN NAME	NUMBER OF STRAINS	FREQUENCY
PROTOTYPE	16	0.2353
IS002	37	0.5441
IS168	8	0.1177
IS172	4	0.0588
1S326	1	0.0147
IS664	1	0.0147
IS768	1	0.0147
TOTAL	68	1.00

TABLE 8: HPV 6 GENOTYPE, VARIANTS AND SUBTYPES

STRAIN NAME	LENGTH	SPECIFIC GENE(S)	NUCLEOTIDE POSITION CHANGES	% HOMOLOGY TO PROTOTYPE	PATIENT POPULATION	# PATIENTS STRAINS FOUND	SOURCE	REFERENCE
HPV 6b, prototype	7902bp Note: 1503bp region is from nucleotides 5789-7291	ALL	NA	NA	NA	NA	condyloma acuminatum	Schwarz *et al.* (1983) EMBO, 2(12), 2341-8
M51033	1503bp	L1	6203, 6217, 6507	99.8	heterosexual & homosexual patients, St. Mary's Hospital, UK	1/17	condyloma acuminatum	Caparrós-Wanderley *et al.* (1999) Journal of General Virology, 80, 1025-33
M44371	1503bp	L1	6598, 6661(C), 7219	99.8	heterosexual & homosexual patients, St. Mary's Hospital, UK	1/17	condyloma acuminatum	Caparrós-Wanderley *et al.* (1999) Journal of General Virology, 80, 1025-33
F49413	1503bp	L1	5923, 6052, 6073, 6217, 6598, 6625, 6661, 7219	99.5	heterosexual & homosexual patients, St. Mary's Hospital, UK	4/17	condylomata acuminata	Caparrós-Wanderley *et al.* (1999) Journal of General Virology, 80, 1025-33

F47718	1503bp	L1	5923, 6052, 6073, 6217, 6598, 6625, 6661, 7079, 7218	99.4	heterosexual & homosexual patients, St. Mary's Hospital, UK	3/17	condylomata acuminata	Caparrós-Wanderley *et al.* (1999) Journal of General Virology, 80, 1025-33
M48364	900bp	L1	5923, 6044, 6052, 6073, 6217, 6598, 7219 (6625, 6661, 6727, 7027, 7079 absent from sequence)	99.4	heterosexual & homosexual patients, St. Mary's Hospital, UK	1/17	condyloma acuminatum	Caparrós-Wanderley *et al.* (1999) Journal of General Virology, 80, 1025-33
M47875	1503bp	L1	5923, 6052, 6073, 6217, 6394, 6598, 6625, 6661, 7205, 7219	99.3	heterosexual & homosexual patients, St. Mary's Hospital, UK	1/17	condyloma acuminatum	Caparrós-Wanderley *et al.* (1999) Journal of General Virology, 80, 1025-33
M46769	1503bp	L1	5923, 6052, 6073, 6094, 6217, 6598, 6625, 6661, 7219, 7245	99.3	heterosexual & homosexual patients, St. Mary's Hospital, UK	1/17	condyloma acuminatum	Caparrós-Wanderley *et al.* (1999) Journal of General Virology, 80, 1025-33
F50027	1503bp	L1	5923, 6052, 6073, 6217, 6598, 6625, 6661, 7079, 7219, 7227	99.3	heterosexual & homosexual patients, St. Mary's Hospital, UK	1/17	condyloma acuminatum	Caparrós-Wanderley *et al.* (1999) Journal of General Virology, 80, 1025-33

F50528	1503bp	L1	5923, 6052, 6073, 6217, 6598, 6625, 6661, 7079, 7219, 7227, 7271	99.3	heterosexual & homosexual patients, St. Mary's Hospital, UK	1/17	condyloma acuminatum	Caparrós-Wanderley *et al.* (1999) Journal of General Virology, 80, 1025-33
F50602	1503bp	L1	5923, 6013, 6052, 6073, 6217, 6598, 6625, 6661(C), 7219, 7254	99.3	heterosexual & homosexual patients, St. Mary's Hospital, UK	1/17	condyloma acuminatum	Caparrós-Wanderley *et al.* (1999) Journal of General Virology, 80, 1025-33
M6	1503bp	L1	5923, 6052, 6073, 6217, 6426, 6478, 6598, 6625, 6661, 7079, 7215	99.3	heterosexual & homosexual patients, St. Mary's Hospital, UK	1/17	condyloma acuminatum	Caparrós-Wanderley *et al.* (1999) Journal of General Virology, 80, 1025-33
M49898	1503bp	L1	5840, 5903, 5923, 6052, 6073, 6214, 6217, 6325, 6598, 6625, 6661, 6727, 7027, 7219	99.1	heterosexual & homosexual patients, St. Mary's Hospital, UK	1/17	condyloma acuminatum	Caparrós-Wanderley *et al.* (1999) Journal of General Virology, 80, 1025-33
Georgia B-5	254bp Note: 254bp region is from nts 6486-6739 of prototype	L1	6598	99.6	Patients from locations worldwide	6/18 (1 Georgia, 4 India, 1 Philippines)	condylomata acuminata	Icenogle *et al.* (1991) Virology, 184, 101-7

Alaska C-36	254bp	L1	6598, 6661	99.2	Patients from locations worldwide	4/18 (1 Alaska, 3 Georgia)	condylomata acuminata	Icenogle *et al.* (1991) Virology, 184, 101-7
Georgia G-4	254bp	L1	6598, 6625	99.2	Patients from locations worldwide	2/18 (2 Georgia)	condylomata acuminata	Icenogle *et al.* (1991) Virology, 184, 101-7
Philippines A-6	254bp	L1	6598, 6625, 6661	98.8	Patients from locations worldwide	6/18 (1 Philippines, 5 Georgia)	condylomata acuminata	Icenogle *et al.* (1991) Virology, 184, 101-7
SN6-11	256bp Note: 256bp region is from nts 7676-7931 of prototype	LCR	7679	99.6	Patients from locations worldwide	8/62 (1 Senegal, 6 India, 1 Japan) 1/62 prototype (German)	cervical swabs and genital warts	Heinzel *et al.* (1995) Journal of Clinical Microbiology, 1746-54
IN6-6	256bp	LCR	7679, 7748,	99.2	Patients from locations worldwide	7/62 (2 India, 3 Japan, 2 New York)	genital warts and laryngeal papillomas	Heinzel *et al.* (1995) Journal of Clinical Microbiology, 1746-54
NY6-16	256bp	LCR	7679, 7747, 7748	98.8	Patients from locations worldwide	1/62 (New York)	laryngeal papilloma	Heinzel *et al.* (1995) Journal of Clinical Microbiology, 1746-54

B6-17	256bp	LCR	7696, 7747, 7748, 7909	98.5	Patients from locations worldwide	2/62 (All Brazil)	cervical scrapes	Heinzel *et al.* (1995) Journal of Clinical Microbiology, 1746-54
B6-15	256bp	LCR	7696, 7747, 7748, 7884	98.5	Patients from locations worldwide	1/62 (Brazil)	cervical swabs and genital warts	Heinzel *et al.* (1995) Journal of Clinical Microbiology, 1746-54
IN6-3	256bp	LCR	7679, 7747, 7748, 7909	98.5	Patients from locations worldwide	6/62 (3 India, 3 New York)	genital warts and laryngeal papillomas	Heinzel *et al.* (1995) Journal of Clinical Microbiology, 1746-54
SN6-1	256bp	LCR	7696, 7748, 7770, 7819, 7897	98.0	Patients from locations worldwide	1/62 (1 Senegal)	cervical swabs	Heinzel *et al.* (1995) Journal of Clinical Microbiology, 1746-54
G6-42	256bp	LCR	7748; 20bp insert between 7812 and 7813	91.8	Patients from locations worldwide	1/62 (Germany)	genital warts	Heinzel *et al.* (1995) Journal of Clinical Microbiology, 1746-54
B6-5	256bp	LCR	7770, 7748; 20bp insert between 7812 and 7813	91.4	Patients from locations worldwide	1/62 (Brazil)	cervical scrapes	Heinzel *et al.* (1995) Journal of Clinical Microbiology, 1746-54

G6-78	256bp	LCR	7748, 7884; 20bp insert between 7812 and 7813	91.4	Patients from locations worldwide	1/62 (Germany)	genital warts	Heinzel *et al.* (1995) Journal of Clinical Microbiology, 1746-54
SN6-3	256bp	LCR	7696, 7748 7770 ; 20bp insert between 7812 and 7813	91.0	Patients from locations worldwide	17/62 (8 Senegal, 3 Germany, 1Brazil, 1 Japan, 4 Italy)	cervical swabs, genital warts and anogenital warts	Heinzel *et al.* (1995) Journal of Clinical Microbiology, 1746-54
SN6-4b (HPV 6c)	256bp	LCR	7696, 7748, 7770, 7884; 20bp insert between 7812 and 7813	90.6	Patients from locations worldwide	2/62 (1 Senegal, 1 German)	cervical swabs and genital warts	Heinzel *et al.* (1995) Journal of Clinical Microbiology, 1746-54
J6-8	256bp	LCR	7696, 7748, 7770, 7911; 20bp insert between 7812 and 7813	90.6	Patients from locations worldwide	1/62 (Japan)	genital warts	Heinzel *et al.* (1995) Journal of Clinical Microbiology, 1746-54
SN6-6b	256bp	LCR	7696, 7748, 7770, 7840, 7884; 20bp insert between 7812 and 7813	90.2	Patients from locations worldwide	1/62 (1 Senegal)	cervical swabs	Heinzel *et al.* (1995) Journal of Clinical Microbiology, 1746-54
6a, 6ma	256bp	LCR	7696, 7748, 7770, 7884, 7911; 20bp insert between 7812 and 7813	90.2	Patients from locations worldwide	2/62 (reference)	vulvar condylomata acuminata	Heinzel *et al.* (1995) Journal of Clinical Microbiology, 1746-54

G6-6	256bp	LCR	7696, 7747, 7748, 7770, 7840, 7884; 20bp insert between 7812 and 7813	89.8	Patients from locations worldwide	5/62 (3 German, 2 New York)	genital warts and laryngeal papillomas	Heinzel *et al.* (1995) Journal of Clinical Microbiology, 1746-54
AM6-1	256bp	LCR	7696, 7747, 7748, 7770, 7840, 7884, 7893; 20bp insert between 7812 and 7813	89.5	Patients from locations worldwide	2/62 (All Amazon)	cervical swabs	Heinzel *et al.* (1995) Journal of Clinical Microbiology, 1746-54
NY6-19	256bp	LCR	7696, 7747, 7748, 7770, 7840, 7884; deletion at 7889; 20bp insert between 7812 and 7813	89.5	Patients from locations worldwide	1/62 (New York)	laryngeal papilloma	Heinzel *et al.* (1995) Journal of Clinical Microbiology, 1746-54
B6-1	256bp	LCR	7696, 7747, 7748, 7769, 7840, 7884, 7893, 7934; 20bp insert between 7812 and 7813	89.1	Patients from locations worldwide	1/62 (Brazil)	cervical scrapes	Heinzel *et al.* (1995) Journal of Clinical Microbiology, 1746-54

HPV 6ert	869bp Note: 869bp is from nts 7151-7890 of prototype	LCR	7219, 7272, 7273, 7282, 7358, 7373, 7523, 7524, 7529, 7534, 7567, 7602, 7606, 7654, 7681, 7860; 94bp insert between 7348 and 7349; 15bp insert between 7419 and 7419; 20bp insert between 7721 and 7722	83.3	NA	1/7	papilloma of the respiratory tract	Chen *et al.* (1996) unpublished
HPV 6e1gt	869bp	LCR	7219, 7272, 7273, 7282, 7358, 7373, 7523, 7524, 7529, 7534, 7538, 7567, 7602, 7606, 7654, 7681, 7860; 94bp insert between 7348 and 7349; 15bp insert between 7419 and 7419; 20bp insert between 7721 and 7722	83.2	NA	1/7	condyloma accuminatum	Chen *et al.* (1996) unpublished

HPV 6e2gt	869bp	LCR	7219, 7272, 7273, 7282, 7334, 7358, 7373, 7523, 7524, 7529, 7534, 7567, 7602, 7606, 7654, 7681, 7860; 94bp insert between 7348 and 7349; 15bp insert between 7419 and 7419; 20bp insert between 7721 and 7722	83.2	NA	4/7	condylomata accuminata and papillomas of the respiratory tract	Chen *et al.* (1996) unpublished
HPV 6f5gt	842bp Note: 842bp is from nts 7151-7890 of prototype	LCR	7219, 7373, 7389, 7560, 7585, 7653, 7678, 7860 ; deletions at 7564, 7682, 7722, 7723; 94bp insert between 7348 and 7349; 3bp insert between nts 7356 and 7357; 7bp insert between nts 7364 and 7365; inserts b/w 7438/7439; 7644/7645; 7657/7658 ; 7662/7663	85.7	NA	1/7	condyloma accuminatum	Chen *et al.* (1996) unpublished

1086	242bp Note: 242bp region is from nts 7289-7436 (148bp)	LCR	7373; 94bp insert between nts 7350 and 7351	99.3 (without insert)	outpatient clinic, STD clinic, labour and delivery at Indiana University Hospitals	2/15	condyloma acuminatum	Roman *et al.* (1995) The Journal of Infectious Diseases, 171, 697-700
1094	242bp	LCR	7358, 7419; 94bp insert between nts 7350 and 7351	98.6 (without insert)	outpatient clinic, STD clinic, labour and delivery at Indiana University Hospitals	3/15	condylomata acuminata	Roman *et al.* (1995) The Journal of Infectious Diseases, 171, 697-700
1082	256bp	LCR	7349, 7358, 7419; 94bp insert between nts 7350 and 7351; 14bp insert between nts 7421 and 7422	98.0 (without inserts)	outpatient clinic, STD clinic, labour and delivery at Indiana University Hospitals	7/15	condylomata acuminata	Roman *et al.* (1995) The Journal of Infectious Diseases, 171, 697-700
1083	256bp	LCR	7358, 7419, 7422; 94bp insert between nts 7350 and 7351; 14bp insert between nts 7421 and 7422	98.0 (without inserts)	outpatient clinic, STD clinic, labour and delivery at Indiana University Hospitals	1/15	condylomata acuminata	Roman *et al.* (1995) The Journal of Infectious Diseases, 171, 697-700

1084	256bp	LCR	7349, 7358, 7419; 94bp insert between nts 7350 and 7351 (change at nt 6(G) of 94bp); 14bp insert between nts 7421 and 7422	98.0 (without inserts)	outpatient clinic, STD clinic, labour and delivery at Indiana University Hospitals	2/15	condylomata acuminata	Roman *et al.* (1995) The Journal of Infectious Diseases, 171, 697-700
W50	734bp Note: 734bp region is from nts 7273 to 104 of prototype	LCR	7373, 7585, 7654, 7860; deletion at 27 and 28; 94bp insert between nts 7350 and 7351(1st 58 nts identical to T70 except nt 50 (T))	82.2	NA	NA	vulvar condyloma	Farr *et al.* (1991) Journal of General Virology, 72, 519-26
T70	734bp	LCR	24bp insert between nts 7323 and 7324 (exact duplication nts 7300-7323); 58bp insert b/w nts 7350 and 7351; 49bp deletion b/w nts 7351 and 7399	86.4	NA	NA	carcinoma of the vulva	Farr *et al.* (1991) Journal of General Virology, 72, 519-26
6d	734bp	LCR	tandem duplication of nts 7528 to 84	-----	NA	NA	Buschke-Lowenstein tumour	Kasher *et al.* (1988) Virology, 165, 225-33

6vc	734bp	LCR	74bp insert between nts 7348 and 7349; 15bp insert between nts 7418 and 7419; 19bp insert between nts 7720 and 7721	85.3	NA	NA	carcinoma of the vulva	Kasher *et al.* (1988) Virology, 165, 225-33

HPV 6a	8010bp	ALL	221, 251, 323, 365, 392, 479, 791, 843, 1011, 1012, 1296, 1426, 1494, 1534, 1535, 1539, 1554, 1669, 1740, 1743, 1867, 1878, 1926, 2193, 2199, 2207, 2314, 2556, 2653, 2709, 2801, 2973, 3147, 3152, 3271, 3387, 3404, 3433, 3460, 3551, 3604, 3642, 3692, 3734, 3764, 3781, 3787, 3793, 3805, 3898, 3934, 4003, 4048, 4136, 4141, 4149, 4194, 4234, 4251, 4275, 4296, 4313, 4322, 4342, 4345, 4346, 4352, 4384, 4386, 4412, 4491, 4578, 4605, 4645, 4646, 4731, 4755, 4869, 4975, 5020, 5025, 5077, 5280, 5406, 5489, 5596, 5619, 5923, 5992, 6052, 6073, 6104, 6598, 6601, 7219, 7358, 7373, 7419, 7524, 7529, 7534, 7567, 7602, 7606, 7653, 7654, 7746, 7790, 7860; insert between nucleotides 20 and 21; 94bp insert between nts 7350 and 7351; 20bp insert between nts 7721 and 7722; 6bp deletion from	97.1	NA	NA	vulvar condyloma acuminatum	Hofmann *et al.* (1995) Virology, 209, 506-18

TABLE 9: HPV 6 FREQUENCY OF L1 REGION STRAINS
(ONLY NUCLEOTIDES 6486 TO 6739 OF PROTOTYPE INCLUDED)

STRAIN NAME	NUMBER OF STRAINS	FREQUENCY
PROTOTYPE	0	0.0000
F49413	18	0.5143
M48364	7	0.2000
Alaska C-36	4	0.1143
Georgia G-4	2	0.0570
M49898	1	0.0286
F50602	1	0.0286
M44371	1	0.0286
M51033	1	0.0286
TOTAL	35	1.00

TABLE 10: HPV 66 GENOTYPE, VARIANTS AND SUBTYPES

STRAIN NAME	LENGTH	SPECIFIC GENE(S)	NUCLEOTIDE POSITION CHANGES	% HOMOLOGY TO PROTOTYPE	PATIENT POPULATION	# PATIENTS STRAINS FOUND	SOURCE	REFERENCE
HPV 66, prototype	7824bp Note: 409 bp region is from nts 6609-7017	ALL	NA	NA	NA	NA	cervical biopsy, CIN I	Delius, H. unpublished
L1AE3	409bp	L1	6699	99.8	500 cervicovaginal samples; patients from Albert Einstein College of Medicine	1/500	cervical scrape specimen	Tachezy *et al.* (1994) Biochemical and Biophysical Research Communications, 204(2), 820-7
L1 protein gene	449bp Note: 449bp region is from nts 6589-7037	L1	6597, 6855, 6997, 7032, 7035, 7036	98.7	Unknown	Unknown	cervical scrape specimen	Bernard *et al.* (1994) Journal of Infectious Diseases, 170(5), 1077-85

TABLE 11: HPV 57 GENOTYPE, VARIANTS AND SUBTYPES

STRAIN NAME	LENGTH	SPECIFIC GENE(S)	NUCLEOTIDE POSITION CHANGES	% HOMOLOGY TO PROTOTYPE	PATIENT POPULATION	# PATIENTS STRAINS FOUND	SOURCE	REFERENCE
HPV 57, prototype	7861bp Note: 297bp region is from nts. 7519-7815	ALL	NA	NA	NA	NA	mucosal lesion	Hirsch-Behnam *et al* (1990). Virus Research, 18(1), 81-97
G32	297bp	LCR (long control region)	81	99.6	German(G), Japan(J), Singapore(S) patients	10/32 All G	wart biopsies (cutaneous)	Chan *et al.* (1997) Virology 239(2): 296-302
J21	297bp	LCR	131, 280	99.3	German(G), Japan(J), Singapore(S) patients	1/32 (J)	wart biopsies (cutaneous)	Chan *et al.* (1997) Virology 239(2): 296-302
J3	297bp	LCR	63, 71, 81, 131, 196, 204, 280	97.6	German(G), Japan(J), Singapore(S) patients	1/32 (J)	wart biopsies (cutaneous)	Chan *et al.* (1997) Virology 239(2): 296-302

J4	297bp	LCR	12, 34, 63, 71, 81, 131, 196, 204, 280	97.0	German(G), Japan(J), Singapore(S) patients	3/32 (All J)	wart biopsies (cutaneous)	Chan *et al.* (1997) Virology 239(2): 296-302
J5	296bp	LCR	63, 71, 81, 98(T), 131, 148, 196, 204, 280, one deletion at 185	96.6	German(G), Japan(J), Singapore(S) patients	8/32 (1G, 5J, 2S)	wart biopsies (cutaneous)	Chan *et al.* (1997) Virology 239(2): 296-302
G44	297bp	LCR	24, 63, 71, 81, 98(T), 105, 131, 147, 148, 150, 196, 204, 280	95.6	German(G), Japan(J), Singapore(S) patients	6/32 (All G)	wart biopsies (cutaneous)	Chan *et al.* (1997) Virology 239(2): 296-302
G46	297bp	LCR	24, 63, 71, 81, 98(C), 105, 131, 147, 148, 150, 196, 204, 280	95.6	German(G), Japan(J), Singapore(S) patients	1/32 (G)	wart biopsies (cutaneous)	Chan *et al.* (1997) Virology 239(2): 296-302
G42	297bp	LCR	24, 63, 71, 81, 98(T), 105, 131, 147, 148, 150, 196, 204, 256, 280	95.3	German(G), Japan(J), Singapore(S) patients	2/32 (All G)	wart biopsies (cutaneous)	Chan *et al.* (1997) Virology 239(2): 296-302
HPV 57b	7868bp Note: 297bp region is from nucleotides 7526-7822	ALL (LCR specific 297bp region)	63, 71, 81, 98, 105, 131, 147, 148, 150, 196, 204, 280, deletion at 24 and 286	95.3	NA	NA	papilloma of the nasal cavity	Wu *et al.* (1993) Lancet, 341(8844), 522-4; Trujillo *et al.* (1996) Virus Genes, 12(2), 165-78; full sequence, unpublished

HPV 57b	7868bp Note: 383bp region is from nucleotides 6335-6717	ALL (L1 specific 383bp region)	3, 39, 72, 82, 90, 114, 126, 135, 141, 180, 189, 201, 252, 288, 321, 357, insertion at 227, deletion at 237	95.3	NA	NA	papilloma of the nasal cavity	Wu *et al.* (1993) Lancet, 341(8844), 522-4; Trujillo *et al.* (1996) Virus Genes, 12(2), 165-78; full sequence, unpublished
partial L1 sequence	383bp Note: 383bp region of prototype is from nts. 6324-6706	L1	3, 6, 15, 26,7, 39, 72, 82, 90, 114, 126, 135, 141, 180, 189, 198, 237, 246, 252, 264, 273, 288, 363, 366, 369, 370, 371, 375, 378, 382	92.4	Renal allograft patient	NA	non-melanoma carcinoma	de Villiers, E.M. unpublished

TABLE 12: HPV 58 GENOTYPE, VARIANTS AND SUBTYPES

STRAIN NAME	LENGTH	SPECIFIC GENE(S)	NUCLEOTIDE POSITION CHANGES	% HOMOLOGY TO PROTOTYPE	PATIENT POPULATION	# PATIENTS STRAINS FOUND	SOURCE	REFERENCE
HPV 58, prototype	7824bp Note: 449bp region is from nucleotides 6588-7036	ALL	NA	NA	NA	NA	cervical cancer specimen	Kirii *et al.* (1991) Virology, 185(1), 425-7
is068	449bp	L1	54	99.8	Patients from 22 countries worldwide and New Mexico	3/9 Brazil, Thailand, Columbia (1/9 prototype)	cervical cancer specimen	Stewart *et al.* (1996) Journal of Virology 70(5), 3127-36
is573	449bp	L1	240	99.8	Patients from 22 countries worldwide and New Mexico	1/9 Paraguay	cervical cancer specimen	Stewart *et al.* (1996) Journal of Virology 70(5), 3127-36
is1021	449bp	L1	211, 240, 294	99.3	Patients from 22 countries worldwide and New Mexico	1/9 Philippines	cervical cancer specimen	Stewart *et al.* (1996) Journal of Virology 70(5), 3127-36
is131	449bp	L1	235, 240, 241	99.3	Patients from 22 countries worldwide and New Mexico	1/9 Bolivia	cervical cancer specimen	Stewart *et al.* (1996) Journal of Virology 70(5), 3127-36

is404	449bp	L1	211, 235, 240, 241	99.1	Patients from 22 countries worldwide and New Mexico	1/9 Mali	cervical cancer specimen	Stewart *et al.* (1996) Journal of Virology 70(5), 3127-36
is417	449bp	L1	105, 110, 124, 211, 235, 240, 241, 429	98.2	Patients from 22 countries worldwide and New Mexico	1/9 Mali	cervical cancer specimen	Stewart *et al.* (1996) Journal of Virology 70(5), 3127-36