Supplemental Online Content

Johnston C, Magaret A, Son H, et al. Viral Shedding 1 Year Following First-Episode Genital HSV-1 Infection. *JAMA.* Published online October 22, 2022. doi:10.1001/jama.2022.19061

eFigures
eTables
eMethods

This supplemental material has been provided by the authors to give readers additional information about their work.

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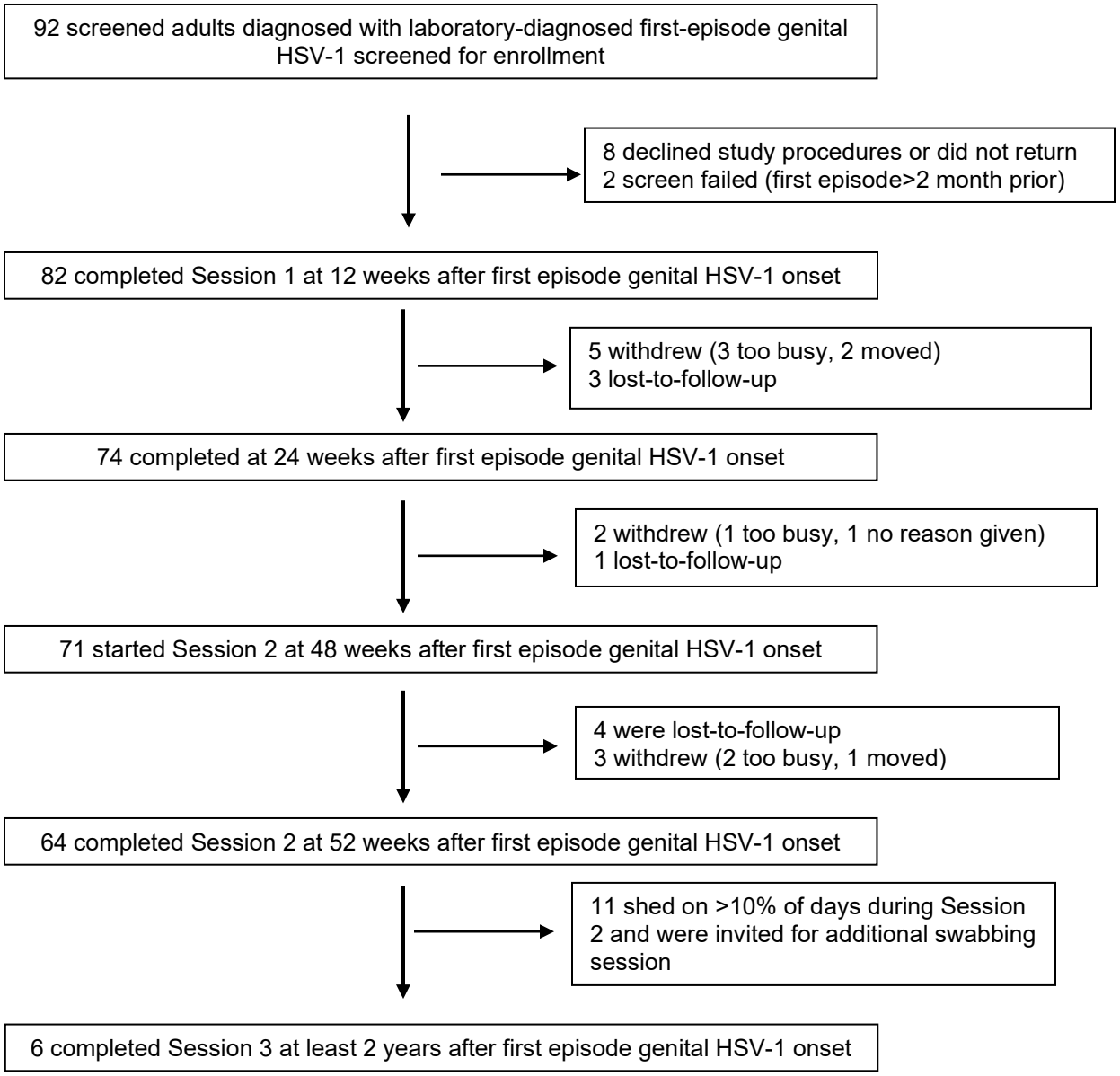
eFigure 1: Participant Flow.



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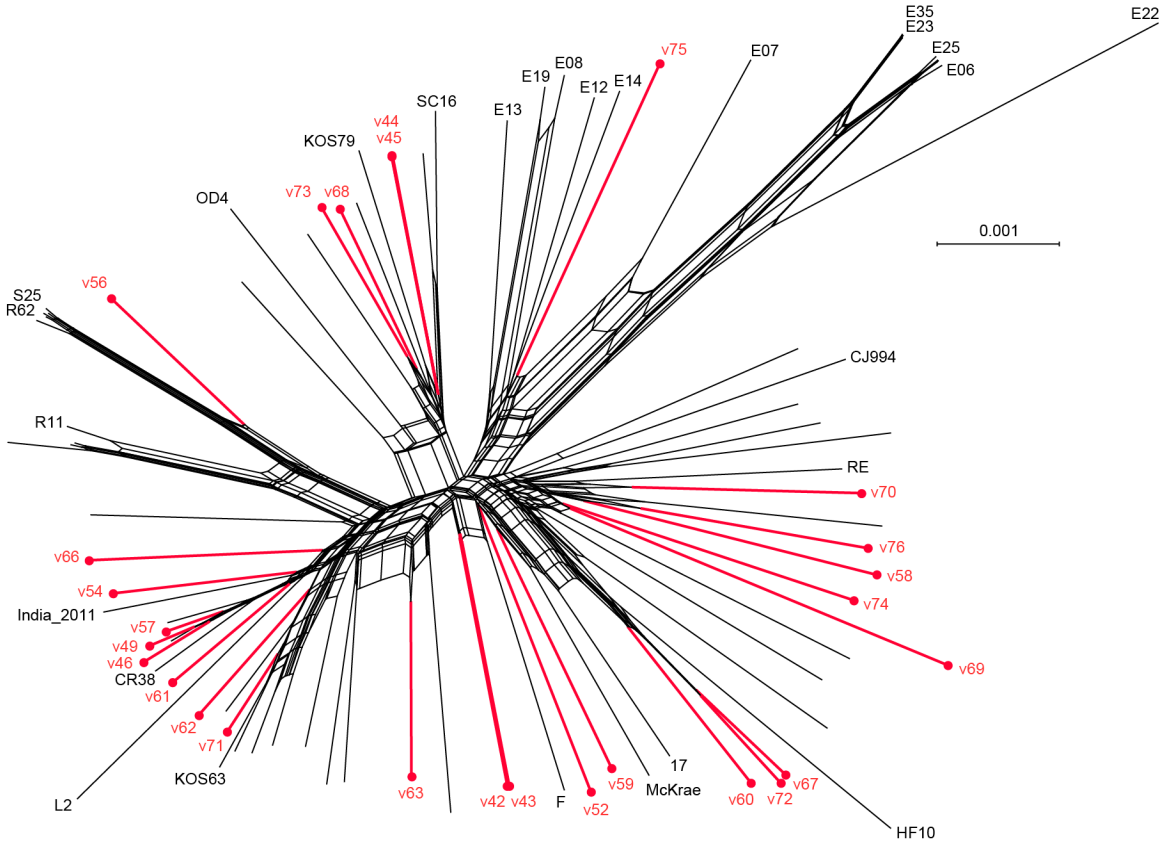
eFigure 2. Genital HSV-1 genomes are distributed among the previously known genetic diversity of HSV-1 genomes. Most previously sequenced HSV-1 genomes were derived from non-genital sources, including herpes labialis (oral), eczema herpeticum (skin), and herpes keratitis (ocular). The network graph of genital HSV-1 genomes from the present study (n=27) are shown in red, with the remaining strains from GenBank in black. Closely paired genomes (v44-v45, v42-v43) were collected from transmission pairs, as previously described[1] SplitsTree (version 4.14.5) was used to create the network graph. See Supplemental Table 1 & 2 for a list of GenBank Accessions for the new and prior genomes.



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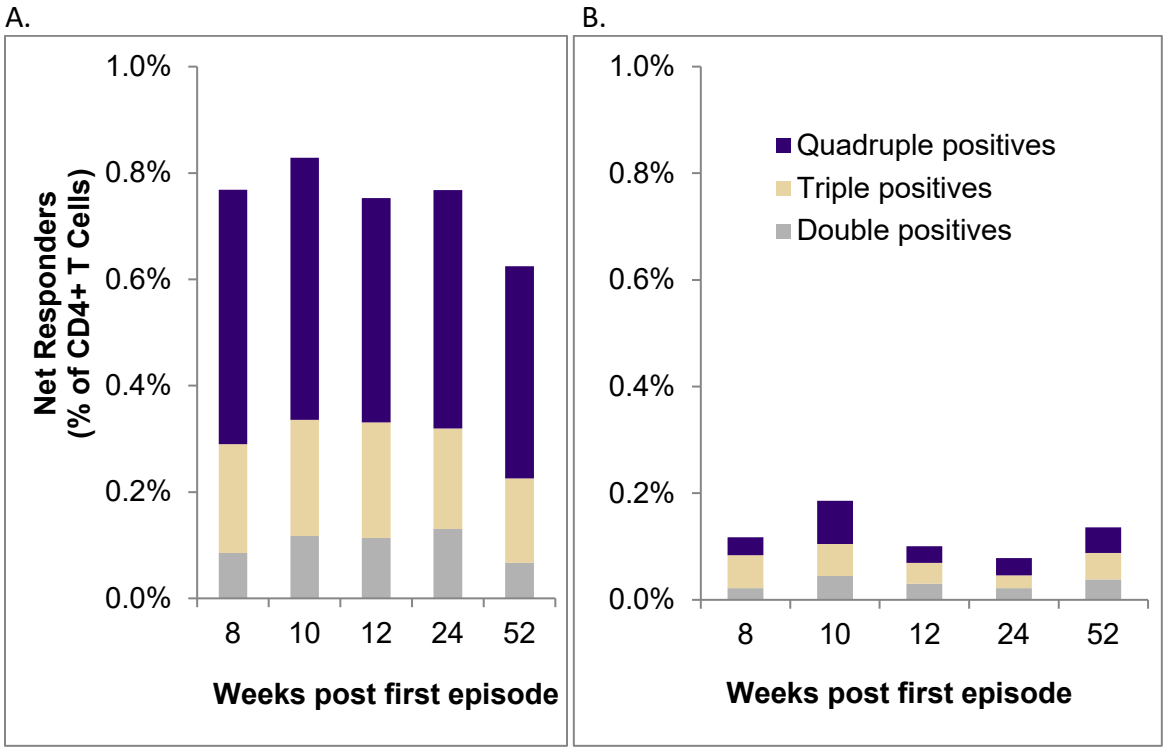
eFigure 3. Representative time course in two participants with primary genital HSV-1 with high (A) and low (B) proportion of HSV-1 specific CD4 T cells in PBMC. Expression of IFN-γ, IL-2, TNF-α and CD40L was tested. Bar colors reflect the sum of possible combinations of net rates for two or three effector molecules of the abundance of CD4 T cells with all 4 responses.



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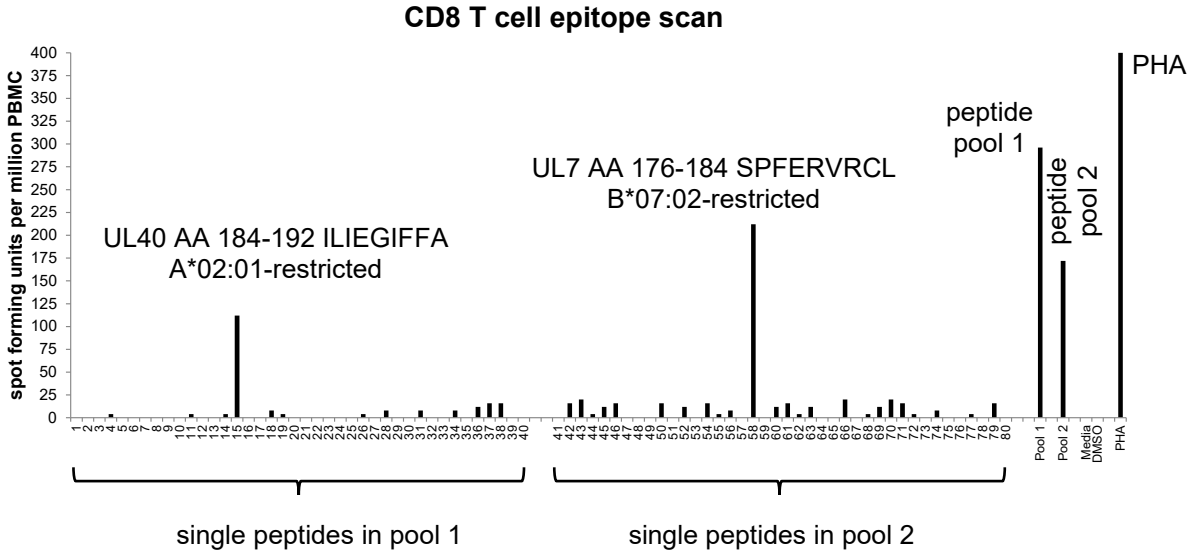
eFigure 4. Representative ELISPOT data from an HLA-A*02:01/HLA-B*07:02 participant in the current study documenting reactivity with peptide pools and individual HSV-1 peptide epitopes. PBMC from representative participant with primary genital HSV-1 infection) were tested in IFN-γ ELISPOT with pooled or single known HSV-1 CD8 T cell peptides at 1 µg/ml concentration. Identity of reactive peptides is indicated. Reactivity to peptide pools 1 and 2, negative controls media and DMSO, and positive control PHA are shown at right.



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eTable 1: 27 new genital HSV-1 genomes from viral cultures

Viral genome ID	Full viral genome name	GenBank Accession	Culture at # days post-primary infection	Infection status at screening
v42	v42_d338_cu_gen_les	OP297869	338	Unable to determine
v43	v43_d17_cu_gen_les	OP297878	17	Primary
v44	v44_d2_cu_gen_les	OP297865	2	Non-primary
v45	v45_d4_cu_gen_les	OP297881	4	Primary
v46	v46_d349_cu_gen_les	OP297868	349	Primary
v49	v49_d257_cu_gen_les	OP297875	257	Primary
v52	v52_d84_cu_gen_les	OP297867	84	Primary
v54	v54_d71_cu_gen_les	OP297873	71	Primary
v56	v56_d6_cu_gen_les	OP297864	6	Primary
v57	v57_d3_cu_gen_les	OP297884	3	Primary
v58	v58_d1_cu_gen_les	OP297870	1	Non-primary
v59	v59_d121_cu_gen_les	OP297876	121	Primary
v60	v60_d3_cu_gen_les	OP297860	3	Primary
v61	v61_d2_cu_gen_les	OP297863	2	Primary
v62	v62_d3_cu_gen_les	OP297872	3	Primary
v63	v63_d284_cu_oral	OP297879	284	Primary
v66	v66_d6_cu_gen	OP297877	6	Primary
v67	v67_d346_cu_gen_les	OP297885	346	Primary
v68	v68_d8_cu_gen_les	OP297880	8	Primary
v69	V69_d2_cu_gen_les	OP297871	2	Unable to determine
v70	v70_d1_cu_gen_les	OP297861	1	Primary
v71	v71_d395_cu_gen_les	OP297866	395	Primary
v72	v72_d53_cu_gen_les	OP297886	53	Non-primary
v73	v73_d4_cu_gen_les	OP297862	4	Primary
v74	v74_d193_cu_gen	OP297874	193	Primary
v75	v75_d23_cu_gen_les	OP297883	23	Non-primary
v76	v76_d266_cu_gen_les	OP297882	266	Primary

eTable 2: HSV-1 genomes used for network graph analysis

Virus Isolate	Country (with location detail, if available)	GenBank Accession #	References
H1211_F-11	Finland	MH999843	[2,3]
H1215_M-15	Finland	MH999846	[2,3]
H12113_ F-13	Finland	MH999842	[3]
H12114_F-14g	Finland	MH999844	[2,3]
H12117_F-17	Finland	MH999845	[2,3]
H12118_F-18g	Finland	MH999847	[2,3]
H1311_F11/	Finland	MH999848	[3]
H1312_M-12	Finland	MH999849	[3]
H1412_F-12g	Finland	MH999851	[3]
H15119_M-19	Finland	MH999850	[3]
SC16	Spain (Madrid)	KX946970	[4]
172_2010	Jena, Germany	LT594105	[5]
2158_2007	Jena, Germany	LT594106	[5]

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3083_2008	Jena, Germany	LT594107	[5]
1319_2005	Germany	LT594108	[5]
270_2007	Manebach, Germany	LT594109	[5]
66_2007	Jena, Germany	LT594110	[5]
1394_2005	Germany	LT594111	[5]
369_2007	Jena, Germany	LT594112	[5]
160_1982	Erfurt, Germany	LT594192	[5]
132_1998	Gelsenkirchen, Germany	LT594457	[5]
L2	Russia (Moscow)	KT780616	[6]
H193	U.S.A.	KT425108	[7]
KOS63	U.S.A. (Houston, TX)	KT425110	[8]
KOS79	U.S.A. (Madison, WI)	KT425109	[8]
CJ994	U.S.A. (Madison, WI)	KR011283	[9]
HSV-1/0116209/India/2011	India	KJ847330	[10]
H166	U.S.A.	KM222726	[11]
H166syn	U.S.A.	KM222727	[11]
RE	New Orleans, U.S.A.	KF498959	n/a
OD4	U.S.A. (Madison, WI)	JN420342	[12]
17	U.K. (Glasgow)	JN555585	[13]
CR38	China (Shenyang)	HM585508	[13]
E06	Kenya (Nairobi)	HM585496	[13]
E07	Kenya (Nairobi)	HM585497	[13]
E08	Kenya (Nairobi)	HM585498	[13]
E10	Kenya (Nairobi)	HM585499	[13]
E11	Kenya (Nairobi)	HM585500	[13]
E12	Kenya (Nairobi)	HM585501	[13]
E13	Kenya (Nairobi)	HM585502	[13]
E14	Kenya (Nairobi)	HM585510	[13]
E15	Kenya (Nairobi)	HM585503	[13]
E19	Kenya (Nairobi)	HM585511	[13]
E22	Kenya (Nairobi)	HM585504	[13]
E23	Kenya (Nairobi)	HM585505	[13]
E25	Kenya (Nairobi)	HM585506	[13]
E35	Kenya (Nairobi)	HM585507	[13]
R11	South Korea (Seoul)	HM585514	[13]
R62	South Korea (Seoul)	HM585515	[13]
S23	Japan (Sapporo)	HM585512	[13]
S25	Japan (Sapporo)	HM585513	[13]
F	U.S.A. (Chicago, IL)	GU734771	[14]
H129	U.S.A. (San Francisco, CA)	GU734772	[14]
McKrae	U.S.A. (Gainesville, FL)	JX142173	[13]
HF10	U.S.A. (New York, NY)	DQ889502	[15]
Ty_25	Japan	MH999840	n/a
Ty_148	Japan	MH999841	n/a
K_86	Japan	MH999839	n/a
K_47	Japan	MH999838	n/a

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eTable 3. Demographic and clinical characteristics of people enrolled by acquisition type.

Baseline Characteristic	Non-primary (n=23)	Primary acquisition (N = 42)	Unknown acquisition (n=17)
Median age, years (range)	25 (19, 57)	26 (16, 64)	29 (19, 47)
Sex, N (%)			
Female	13 (57%)	31 (74%)	10 (59)
Male	10 (43%)	11 (26%)	7 (41%)
Race, N (%)			
White	19 (83%)	36 (86%)	11 (65%)
Black	0 (0%)	0 (0%)	1 (6%)
Asian	0 (0%)	0 (0%)	2 (12%)
Other	1 (4%)	2 (5%)	0 (0%)
Mix/multiple	3 (13%)	4 (10%)	3 (18%)
Median days since genital HSV acquisition at enrollment, if known (range)	58 (46, 70) [n=23]	58 (54, 95) [n=42]	63 (57, 119) [n=17]
Hx of oral HSV	5 (22%)	3 (7%)	2 (12%)
Median days since oral HSV acquisition, if known (range)	765 (58, 5127) [n=5]	3058 (54, 7538) [n=4]	2302 (71, 4532) [n=2]

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eTable 4. Bivariable (B) and multivariable (M)[a] risk factors associated with genital lesions

Effect	Model-predicted Shedding rate (95% CI)	Regressions		
		Model	Risk ratio (95% CI)	p-value
1st session	0.007 (0.003, 0.015)	B	Ref	--
2nd session	0.014 (0.007, 0.026)	B	1.96 (0.77 to 4.98)	0.15
Non-primary/unknown acquisitions	0.003 (0.001, 0.007)	B	Ref	--
Primary acquisitions	0.021 (0.011, 0.038)	B	6.50 (2.37, 17.8)	<0.001
Female	0.012 (0.007, 0.022)	B	Ref	--
Male	0.006 (0.002, 0.016)	B	0.52 (0.17 to 1.61)	0.25
Age < 26	0.014 (0.007, 0.027)	B	Ref	--
Age ≥ 26	0.007 (0.003, 0.015)	B	0.50 (0.18, 1.39)	0.18
1st session among non-primary	0.002 (0.001, 0.006)	M	Ref	--
2nd session among non-primary	0.004 (0.015, 0.012)	M	1.83 (0.57 to 5.86)	0.30
1st session among primary	0.015 (0.006, 0.035)	M	Ref	--
2nd session among primary	0.028 (0.014, 0.060)	M	1.95 (0.71 to 5.37)	0.19
Non-primary during 1st session	0.002 (0.001, 0.006)	M	Ref	--
Primary during 1st session	0.015 (0.006, 0.035)	M	6.21 (1.72 to 22.4)	0.006

[a] For this analysis, those with unknown acquisition type are grouped with non-primaries. For the comparison between first and second session, the model does not distinguish primary from non-primary or unknown acquisition type. For the comparison between non-primary unknown and primary the model does not distinguish 1st session from 2nd session. The **multivariable model** included an interaction term between session and acquisition type. In **multivariable models** including both age and gender, neither age nor gender contributed to the model in estimating shedding frequencies, so those measures were removed in backward elimination

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eTable 5. Bivariable (B) and multivariable (M) risk factors associated with genital and oral HSV-1 shedding among 70 people who did not receive suppressive antiviral therapy between Session 1 and Session 2. For this analysis, those with unknown acquisition type are grouped with non-primaries. For the comparison between first and second session, the model does not distinguish primary from non-primary or unknown acquisition type. For the comparison between non-primary unknown and primary the model does not distinguish 1st session from 2nd session. The multivariable model included an interaction term between session and acquisition type. In multivariable models including both age and gender, neither age nor gender contributed to the model in estimating shedding frequencies, so those measures were removed in backward elimination.

Genital Shedding				
Effect	**Model-predicted Shedding rate (95% CI)**	**Regressions**		
		Model	**Risk ratio (95% CI)**	**p-value**
1st session	0.054 (0.036, 0.083)	B	Ref	--
2nd session	0.028 (0.014, 0.054)	B	0.52 (0.26, 1.01)	0.05
Non-1° acquisitions	0.025 (0.014, 0.045)	B	Ref	--
1° acquisitions	0.079 (0.049, 0.126)	B	3.14 (1.47, 6.73)	0.004
Female	0.053 (0.034, 0.084)	B	Ref	--
Male	0.030 (0.014, 0.063)	B	0.57 (0.24, 1.35)	0.19
Age < 26	0.060 (0.035, 0.100)	B	Ref	--
Age ≥ 26	0.032 (0.018, 0.057)	B	0.54 (0.25, 1.17)	0.12
1st session among non-1°	0.031 (0.016, 0.058)	M	Ref	--
2nd session among non-1°	0.016 (0.006, 0.043)	M	0.53 (0.20, 1.41)	0.20
1st session among 1°	0.010 (0.060, 0.166)	M	Ref	--
2nd session among 1°	0.051 (0.022, 0.118)	M	0.51 (0.21, 1.23)	0.13
non-1° during 1st session	0.031 (0.016, 0.058)	M	Ref	--
1° during 1st session	0.100 (0.060, 0.166)	M	3.25 (1.44, 7.35)	0.005
Oral Shedding				
1st session	0.015 (0.008, 0.027)	B	Ref	--
2nd session	0.016 (0.008, 0.031)	B	1.08 (0.50, 2.37)	0.83
Non-1° acquisitions	0.008 (0.004, 0.016)	B	Ref	--
1° acquisitions	0.029 (0.015, 0.055)	B	3.53 (1.41, 8.81)	0.008
Female	0.015 (0.009, 0.026)	B	Ref	--
Male	0.016 (0.006, 0.039)	B	1.06 (0.37, 3.04)	0.91
Age < 26	0.021 (0.012, 0.037)	B	Ref	--
Age ≥ 26	0.011 (0.005, 0.024)	B	0.51 (0.19, 1.37)	0.18
1st session among non-1°	0.007 (0.003, 0.017)	M	Ref	--
2nd session among non-1°	0.009 (0.003, 0.031)	M	1.29 (0.26, 6.40)	0.75

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1st session among 1°	0.028 (0.013, 0.060)	M	Ref	--
2nd session among 1°	0.029 (0.013, 0.065)	M	1.04 (0.44, 2.47)	0.93
non-1° during 1st session	0.007 (0.003, 0.017)	M	Ref	--
1° during 1st session	0.028 (0.013, 0.060)	M	3.87 (1.22, 12.25)	0.022

eTable 6. Association between shedding rates and polyfunctional cytokine expression

Spearman correlations (two-sided p-value)			% Cytokine expressed		
			At least 2	At least 3	4
Shedding rates	Genital	1st session	-0.09 (0.68)	-0.12 (0.61)	-0.11 (0.61)
		2nd session	0.46 (0.06)	0.29 (0.25)	0.25 (0.31)
	Oral	1st session	0.07 (0.76)	0.06 (0.77)	0.12 (0.58)
		2nd session	0.32 (0.20)	0.34 (0.17)	0.36 (0.15)

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eTable 7. HSV-1 peptides found to be immunogenic in IFN-γ ELISPOT in one or more subjects in this study.		
peptide (HSV-1 open reading frame_amino acids)	**sequence**	**HLA restricting allele**
UL40_184-192	ILIEGIFFA	A*02:01
UL48_479-488	FTDALGIDEY	A*01:01
UL49_281-290	RPTERPRAPA	B*07:02
UL27_17-25	ALLGLTLGV	A*02:01
UL27_561-569	RMLGDVMAV	A*02:01
UL46_702-710	ALSALLTKL	A*02:01
UL46_226-234	AYVSVLYRW	A*24:02
RL2_698-706	VPGWSRRTL	B*07:02
UL7_176-184	SPFERVRCL	B*07:02
UL47_286–294	FLADAVVRL	A*02:01
UL47_544–552	RLLGFADTV	A*02:01
UL48_90-99	SALPTNADLY	A*01:01

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eMethods

Viral culture expansion, nucleocapsid DNA isolation, and deep sequencing

A viral master stock was created from each culture-positive swab (27 total; see Supplemental Table 1 below), by expansion on Vero (African green monkey kidney) cells (ATCC, CCL-81). The titer of each stock was determined by limiting dilution on Vero cell monolayers under methylcellulose. To collect viral nucleocapsid DNA, each master stock was used to infect Vero cells at an MOI of 5. From this infection, DNA was isolated according to previously described methods. using Freon-based separation, proteinase K digestion, phenol-chloroform DNA extraction, and ethanol precipitation[16]. Viral nucleocapsid DNA was sheared on a Covaris M220 (parameters: 60-s duration, peak power of 50, 10% duty cycle, 4°C) and used to create barcoded Illumina TruSeq DNA sequencing libraries according to manufacturer's protocols. Libraries were checked by Qubit (Invitrogen, CA), Bioanalyzer (Agilent), and quantitative PCR (KAPA Biosystems), before paired-end sequencing (2 × 300 bp; v3 chemistry) on our in-house Illumina MiSeq.

De novo assembly and network graph analysis of viral genomes

First, HSV-specific reads were selected from by BLAST-based comparison of all Illumina sequence data (FASTQ files) against a database of all HSV genes and genomes in GenBank. The resulting sequence reads were quality-controlled using our published Viral Genome Assembly (VirGA) pipeline[17], which includes adaptor trimming via Trimmomatic[18], and removal of low quality bases (minimum Phred score 30, over a 15 bp window size), short read fragments (minimum size 30 bp), and unpaired reads. The resulting paired-end reads were used for viral genome *de novo* assembly via MetaSpades v.3.14.0 (parameters: *spades.py -k 21, 33, 55, 77 --meta -1 $R1 -2 $R2*)[19]. The resulting MetaSpades contigs were compiled into full-length consensus genomes using VirGA, and annotated by comparison to the HSV1 reference genome (strain 17, GenBank JN555585)[17,20]. These 27 viral genomes were compared to a globally-representative set of 60 previously sequenced viral genomes[3] (see Supplemental Table 2 for strain names, source locations, GenBank accessions, and references). Trimmed viral genomes (excluding the terminal copies of the repeat regions) were aligned using

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MAFFT v7.394 with default parameters[21]. Network graphs were constructed using SplitsTree v4 (version 4.14.5; uncorrected P-distance, gaps excluded)[22].

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