Epidemiol. Infect. (2017), **145**, 2618–2625. © Cambridge University Press 2017
doi:10.1017/S0950268817001509

Differentiation between wild-type and vaccines strains of varicella zoster virus (VZV) based on four single nucleotide polymorphisms

L. JIN[1]*†, S. XU[2]†, P. A. C. MAPLE[1]‡, W. XU[2] AND K. E. BROWN[1]

[1] *Virus Reference Department, National Infections Service, Public Health England, London, UK*
[2] *Institute of Viral Disease Control and Prevention, Chinese Centers for Disease Control and Prevention, Beijing, China*

Received 24 October 2016; Final revision 18 May 2017; Accepted 28 June 2017; first published online 27 July 2017

Summary

Varicella–zoster virus (VZV) infection (chickenpox) results in latency and subsequent reactivation manifests as shingles. Effective attenuated vaccines (vOka) are available for prevention of both illnesses. In this study, an amplicon-based sequencing method capable of differentiating between VZV wild-type (wt) strains and vOka vaccine is described. A total of 44 vesicular fluid specimens collected from 43 patients (16 from China and 27 from the UK) with either chickenpox or shingles were investigated, of which 10 had received previous vaccination. Four sets of polymerase chain reactions were set up simultaneously with primers amplifying regions encompassing four single nucleotide polymorphisms (SNPs), '69349-106262-107252-108111'. Nucleotide sequences were generated by Sanger sequencing. All samples except one had a wt SNP profile of 'A-T-T-T'. The sample collected from a patient who received vaccine 7–10 days ago, along with VZV vaccine preparations, Zostavax and Baike-varicella gave a SNP profile '*G-C-C-C*'. The results show that this method can distinguish vaccine-derived virus from wt viruses from main four clades, (clades 1–4) and should be of utility worldwide.

Key words: Four-SNPs profile, varicella–zoster virus (VZV), wild-type and vaccine strains.

INTRODUCTION

Varicella–zoster virus (VZV) causes varicella (chickenpox), a contagious human infectious disease characterised by a generalised vesicular rash. Subsequent to primary infection, lifelong latency is established in the sensory nerve ganglia and reactivation may occur years or decades later to cause herpes zoster (shingles) under conditions of declining immunity and/or with increasing age [1, 2].

* Author for correspondence: Dr L. Jin, Virus Reference Department, National Infections Service, Public Health England, 61 Colindale Avenue, London NW9 5EQ, UK.
(Email: li.jin@phe.gov.uk)
† These authors are co-first authors.
‡ Current address: Division of Clinical Neuroscience, Queen's Medical Centre, University of Nottingham, Nottingham, UK.

VZV belongs to the family *Herpesviridae*, subfamily *Alphaherpesvirinae*. It is an enveloped virus and consists of an approximately 125 kb of linear, double stranded DNA genome enclosed by an icosahedral nucleocapsid, which contains 71 open reading frames (ORFs), three of which are duplicated in the inverted repeat regions. Two main coding regions, one unique long (U_L) and one unique short (U_S), each bounded by inverted repeat regions and five repeat regions (R1–R5) have been identified in the genome [3, 4].

The VZV genome is highly conserved with an estimated mutation rate of 10^{-6}–10^{-7}/site/year [5]. Five clades (1–5) and two provisional clades (VI and VII) have been identified based on an internationally agreed genotyping scheme [6]. The universal

nomenclature enables the interchange and comparison of genotyping data worldwide. Genotypic differentiation of the clades can be achieved using a panel of 16 single nucleotide polymorphisms (SNPs) scattered across the whole genome [5]. It is known that historically clades 1 and 3 predominate in Europe, clade 2 is found in Japan, China and Korea, and clades 4 and 5 are found mainly in India, Nepal, Bangladesh and Africa [7–10]. Small numbers of possible novel genotypes have been designated as provisional clades: clade VI strains were found in France, Italy, USA and Australia [10], a clade VII strain was isolated in the USA in 2002 [11] and clade VIII and IX strains have been reported in Germany [12]. All the VZV vaccine strains, apart from one formulation in Korea, are based on the Oka strain of VZV isolated from a child in Japan [13] and are clade 2 viruses.

Neither England nor China offers routine vaccination of children against varicella although the vaccine is licensed in both countries. Currently, in China, population-based varicella vaccination is not undertaken as national policy; however, vaccination is available on a private basis for children aged 12–18 months. From 1 September 2014 a shingles vaccination program for older adults has been launched in England with the aim of reducing the incidence and severity of shingles in those targeted. The English shingles vaccination program offers Zostavax (Sanofi Pasteur MSD, France), a live attenuated vaccine, to adults aged 70 and currently there is a catch up programme for those aged 78 and 79 [14, 15]. The Zostavax vaccine used is derived from the Oka strain of VZV (vOka) and has significantly higher antigen content than the Varivax (Sanofi Pasteur MSD, France) or Varilrix (GlaxoSmithKline, UK) varicella vaccine used for vaccination against chickenpox.

Cases of both local vaccine-related rash and disseminated rash have occurred following immunisation with both varicella or zoster vaccine, especially if inadvertently given to immunocompromised individuals and it has been shown that the vaccine virus can establish latency with subsequent reactivation [16]. Therefore, as part of the roll out of the shingles vaccination programme it has been recommended that all cases of local or disseminated zoster following vaccination be forwarded to the national laboratory at Public Health England, Colindale to determine whether the VZV isolated is wild-type (wt) or vaccine derived [14].

Differentiation of VZV vaccine strains from wt strains has become important in VZV surveillance for mainly three reasons: monitoring side reactions of vaccination, e.g. rashes and shingles occurring in vaccinated individuals; assessing effectiveness of vaccination by measuring national incidences of varicella and shingles; and identifying the frequency that the attenuated vOka can establish latency and subsequently reactivate to cause disease. Characterisation of wt VZV and the Oka vaccine strains can be achieved only by molecular genotyping methods. In previous studies, the genotypic differentiations of vOka from wt VZV strains were based mainly on SNPs 69349 in ORF38 [17, 18] or 105705 or 106262 in ORF62 [19–24]. However, a number of studies have shown that vOka vaccine preparations contain a mixture of virus strains leading to the recommendation that a set of four-markers in ORF62 should be used for diagnostic differentiation of wt VZV and vaccine-type VZV [25, 26].

In this study, an amplicon-based approach for distinguishing vOka from all wt VZV strains has been developed using four SNPs including three in ORF62, which were recently recommended [26] and one in ORF38 that has proved useful in the USA [CDC SOP, Dr DS Schmid, personal communication]. The methodology established was verified using samples from clades 1 to 4 collected from patients in the UK and China and confirm that this approach should have worldwide utility.

METHODS

Study populations

In this study, 44 vesicular fluid/swab specimens were investigated. The 28 specimens from 27 UK patients were received, including 17 for the purpose of chickenpox or shingles diagnosis and 10 enrolled as part of Public Health England shingles surveillance in patients over 70 (Table 1). Most (25/27) UK patients presented with shingles and 10 of them had been vaccinated with Zostavax (Sanofi Pasteur MSD, France) and one, a child had been vaccinated with the Varivax (Sanofi Pasteur MSD, France). The 16 samples collected from Chinese patients comprised 11 chickenpox samples and five shingles samples. Nine of the samples had previously been genotyped [27] and belonged to clades 2 and 4 (Table 1). The vaccination histories of Chinese patients were unknown, but routine varicella and shingles vaccination is not offered in China.

Vaccine preparations and control strains used

Wild-type Dumas strain was obtained from the European Collection of Cell Cultures (Porton Down,

https://doi.org/10.1017/S0950268817001509 Published online by Cambridge University Press

Table 1. *Patients and clinical specimens investigated in the study*

Case ID	Age, gender	Clinical symptom	Rash onset post-vaccination	Location	4-SNPs result	Genotype result
UK-1	18 years, M	Shingles	Unvaccinated	England	Wild	Not done
UK-2	19 years, F	Shingles	Unvaccinated	England	Wild	Not done
UK-3	57 years, F	Shingles[a,b]	Unvaccinated	England	Wild	Not done
UK-4	1 years, F	Chickenpox	Unvaccinated	England	Wild	Not done
UK-5	44 years, M	Shingles[b]	Unvaccinated	England	Wild	Not done
UK-6	68 years, M	Shingles	Unvaccinated	England	Wild	Not done
UK-7	78 years, F	Shingles[a]	Yes (9 days)	England	Wild	Not done
UK-8	79 years, M	Shingles	Yes (9 days)	England	Wild	Not done
UK-9	70 years, F	Shingles	Yes (7 days)	England	Wild	Not done
UK-10	72 years, F	Shingles[b]	Yes (14 months)	England	Wild	Not done
UK-11	78 years, F	Shingles[a,b]	Yes (4 months)	England	Wild	Not done
UK-12	3 years, M	Chickenpox	Yes (7 days)	England	Wild	Not done
UK-13	72 years, F	Shingles[b]	Yes (20 months)	England	Wild	Not done
UK-14	78 years, M	Shingles	Yes (3 days)	England	Wild	Not done
UK-15	70 years, F	Shingles	Yes (2 days)	England	Wild	Not done
UK-16	56 years, M	Shingles	Unvaccinated	England	Wild	Clade 3
UK-17	65 years, F	Shingles[a]	Unvaccinated	England	Wild	Clade 1
UK-18	76 years, F	Shingles[a,b]	Unvaccinated	England	Wild	Clade 1
UK-19	65 years, F	Shingles[a]	Unvaccinated	England	Wild	Clade 1
UK-20	55 years, F	Shingles[a]	Unvaccinated	England	Wild	Clade 1
UK-21	90 years, M	Shingles[a,b]	Unvaccinated	England	Wild	Clade 1
UK-22	70 years, M	Shingles[a]	Unvaccinated	England	Wild	Clade 1
UK-23-1			Unvaccinated			
23-2	50 years, M	Shingles[a]		England	Wild	Clade 3
UK-24	70 years, F	Shingles	Yes (1 day)	England	Wild	Not done
UK-25	3 years, M	? Shingles[a]	Unvaccinated	England	Wild	Not done
UK-26	55 years, F	Varicella rash	Unvaccinated	England	Wild	Not done
UK-27	71 years, M	Varicella like rash	Yes (7–10 days)	Scotland	Vaccine	Not done
JL14-2	2 years, F	Chickenpox	Unknown	China	Wild	Clade 2
JL14-4	10 years, M	Chickenpox	Unknown	China	Wild	Clade 2
JL14-5	21 years, M	Chickenpox	Unknown	China	Wild	Clade 2
JL14-6	11 years, M	Chickenpox	Unknown	China	Wild	Clade 2
JL14-8	11 years, F	Chickenpox	Unknown	China	Wild	Clade 2
JL14-9	20 years, F	Chickenpox	Unknown	China	Wild	Clade 2
JL14-13	18 years, F	Chickenpox	Unknown	China	Wild	Clade 2
QH13002	8 years, M	Chickenpox	Unknown	China	Wild	Clade 2[c]
QH13003	12 years, F	Chickenpox	Unknown	China	Wild	Clade 2[c]
QH13008	17 years, M	Chickenpox	Unknown	China	Wild	Clade 2[c]
QH13009	11 years, M	Chickenpox	Unknown	China	Wild	Clade 2[c]
GD-09-14	53 years, M	Shingles	Unknown	China	Wild	Clade 2[c]
GD-09-20	50 years, M	Shingles	Unknown	China	Wild	Clade 2[c]
GD-09-21	64 years, F	Shingles	Unknown	China	Wild	Clade 4[c]
GD-09-22	56 years, M	Shingles	Unknown	China	Wild	Clade 2[c]
GD-09-23	55 years, M	Shingles	Unknown	China	Wild	Clade 2[c]

[a] Patient with immunosuppression.
[b] Previous shingles.
[c] Genotype/clade result from previous study [27].

UK). ROD VZV qDNA with a known concentration of 6×10^5 genomic copies/ml was used as a positive control for the real-time polymerase chain reaction (PCR) and was purchased from Source Bioscience (Nottingham, UK). The vaccine preparations, Zostavax (Sanofi Pasteur MSD, France) and Baike-varicella (Changchun BCHT Biotechnology Co., China) were used as sources of vOka. The Zostavax vaccine contained 19 400 P PFU/dose and the Baike-varicella vaccine contained 3300–4000 PFU/dose. These control

https://doi.org/10.1017/S0950268817001509 Published online by Cambridge University Press

Table 2. *Primers designed for detection of the four SNPs*

ORF	SNP	Primer	Primer sequence 5′-	Amplicon size (SNP/size excluding primers)
38	69349	vzv49f	TGAGCAACTTGATCCGTGTC	347 bp (190/307 bp)
		vzv49r	CCTCGCCATAAAGCCACTAC	
62	106262	vzv62f	ACAAACACAGGGGTTGTTCG	339 bp (194/299 bp)
		vzv62r	GCGTTTTATTACTGTCGACC	
	107252	vzv52f	CGGTGGACACACAGAAAGAG	360 bp (121/320 bp)
		vzv52r	GCCCTGAACCAGTTCTACCA	
	108111	vzv11f	ACCCACCATCATTTGAGTCC	350 bp (127/310 bp)
		vzv11r	GCCGTTTGAGACCGATGATA	

strains were reconstituted and diluted using sterile nuclease-free water before DNA extraction.

DNA extraction

DNA extraction was carried out directly from 100 µl diluted vesicle fluid or diluted control samples using the automated BioMerieux NucliSENSeasyMAG system (Biomérieux, France) for quantitative real-time PCR testing. For the purpose of genotyping 200 µl vesicle fluid was extracted using the QIAamp DNA Mini kit (Qiagen, Valencia, CA) according to the manufacturer's instruction. The eluted DNA (50 µl) was stored at −30 °C before PCR amplification.

Detection of VZV DNA

All patient samples were initially confirmed VZV DNA positive by real-time PCR, which targeted a 77-base pair (bp) region of ORF29 in the VZV genome [28]. The assay was slightly modified; 2·5 µl of extracted sample was amplified with 12·5 µl of 2× QuantiTect Probe PCR Master Mix (Qiagen), 25 pmol of both forward and reverse primers and 5 pmol of the probe in a total reaction volume of 25 µl. The real-time PCR was performed using an ABI 7500 real-time PCR system (Applied Biosystems).

Genotypic discrimination of wt VZV and vOka vaccines

The genotyping methodology for distinguishing between vaccine and wt strains was based on four previously reported SNPs [26]. Three of the SNPs were located within ORF62 at positions 106262, 107252, 108111 and the fourth one at 69349 in ORF38. Four pairs of primers for the PCRs and sequencing were designed (Table 2) with software Primer 3 [29] and BLAST searched for confirmation. Four separate PCR reaction mixtures were set up simultaneously using Katara Ex Taq™ kit (RR001A). Each contained 5 µl of 10× Ex-Taq buffer, 4 µl of 2·5 mM Ex-dNTP mix and 0·25 µl of Ex-Taq polymerase, in addition to 20 pmol of each primer and 5–10 µl sample DNA to make up to 50 µl with sterile nuclease-free water. The reactions were processed for 30 cycles of 40 s at 94 °C, 40 s at 55 °C and 1·5 min at 72 °C after an initial denaturation step for 2 min at 95 °C, and followed by extension at 72 °C for 5 min. PCR products were visualised by electrophoresis in a 2% agarose E-gel (Invitrogen). A positive result should display amplicon bands of molecular size 339–360 bp depending on the primer pairs used (Table 2).

Confirmation of VZV clades

To confirm that the assay could distinguish vaccine from all four major clades VZV clade identification was carried out as previously described [6, 7]. Five SNPs in positions 37902, 38019, 38055, 38081 and 38177 within a 447 bp region of ORF22 (Dumas, GenBank No. X04370) were detected to identify all clades except for the clades 1 and 3; then the 6th SNP in position 39394 of ORF22 were further sequenced to discriminate between clades 1 and 3 (Table 3).

Clean-up of PCR amplicons was performed using Agencourt AMPure XP PCR purification kit (Auto Q Biosciences Ltd, Berkshire, UK) and Sanger sequencing undertaken by the PHE Genomic Services Unit. Sequence data were analysed using an appropriate software program SeqMan (DNAStar).

RESULTS

Differentiation between vaccine and wt strains by the 4-SNPs method

The 4-SNPs assay for differentiation between vaccine and wt strains was evaluated by testing the wt Dumas and vaccine vOka strains from both Zostavax and

https://doi.org/10.1017/S0950268817001509 Published online by Cambridge University Press

Table 3. *SNP patterns for classification of VZV clades [6, 12]*

Clade	Residue in ORF22 (447 bp)					ORF22 (6th SNP)
	37902	38019	38055	38081	38177	39394
1	A	G	T	A	G	**G**
2	G	G	C	C	A	A
3	A	G	T	A	G	**A**
4	A	A	C	C	A	G
5	A	G	T	C	G	A
VI	A	G	T	C	A	A
VII	G	G	T	C	G	Unknown
VIII	A	G	T	C	A	A
IX	A	G	C	A	G	G

Baike-varicella. The 4-SNPs, '69349–106262–107252–108111' were confirmed as identical with those previously reported respectively, a wt SNP profile 'A–T–T–T' (GenBank accession number X04370 for Dumas strain and AB097933 for pOka strain) or vaccine SNP profile 'G–C–C–C' (GenBank accession number AB097932 for vOka strain). Whereas the results in a 1 : 1 mixture of wide: vaccine adjusted based on the real-time CT value showed a mixed profile '*G–C*–T–T' repeatedly, targeted during PCR amplifications that two wt SNPs and two vaccine SNPs were detected (Table 4).

VZV characterisation in clinical specimens and Rod VZV DNA

All clinical specimens, except for UK-27 (Table 1) plus the Rod strain tested had the profile 'A–T–T–T', belonging to wt VZV. One sample from a HIV positive patient (case UK-23) who had two vesicular fluids tested, showed A–T–T–T whereas the second sample showed A–T–C–T. The SNP 107252 was identical to that of the vaccine strain; however, it became T after re-testing by PCR and sequencing. Sample UK-27 was collected from a 71-year-old patient who presented varicella like rash 7–10 days post-vaccination, showed vaccine SNP profile G–C–C–C. In addition to the 44 samples, five vesicular fluids collected from patients previously vaccinated with Zostavax were untypable by this approach due to their low DNA content (real-time PCR CT >30).

Identification of VZV clades

Due to the available volume of samples, 15 (eight from the UK and seven from China) specimens were further genotyped. Including the nine Chinese samples genotyped previously [27], there were six clade 1, 15 clade 2, two clade 3 and one clade 4 samples. All clade 1 and 3 samples were found in England and the clade 2 and 4 samples were found in China (Table 1).

DISCUSSION

Following the introduction of the vaccine programme, in order to determine the long-term consequences if vaccine strain persists and transmission from varicella vaccine recipients, a simple and reliable assay is needed, which reliably differentiates between wt VZV strains and vOka vaccine strains in clinical specimens as a part of the enhanced shingles in England. Recent studies reported that the wt allele has been shown to be present in a small percentage (2–6%) of genomes in the Oka vaccine preparation [25, 26] thus using just one of the loci is not reliable for discrimination between vOka and wt VZV and a minimum of three vaccine markers (106262, 107252 and 108111) in ORF62 must be used all together. In order to provide a simple and definitive assay for directly testing clinical specimens, we developed a PCR-Sanger sequencing-based approach, which targets the three markers recently recommended [26], and an additional one at position 69349 in ORF38. The fourth SNP 69349 in ORF38 was chosen because the nucleotide A creates a *PstI* restriction site in most wt strains, including clades 1, 3, 4, 5, IV and VII and 70% of clade 2 strains but the substitute G detected only in vOka and 30% of clade 2 strains abolishes this *PstI* site [18, 26, 30].

Although limited clinical specimens were tested, the approach reported here has been validated and shown to be capable of differentiating between vaccine and wt VZV strains from different geographical locations. In our study, 11 patients were recently vaccinated who presented with shingles (10 patients) or chickenpox (one patient). The samples were collected over a period of between 2 days and 20 months after vaccination. Only one vaccine-type VZV was detected (9·1%), of which the patient (UK-27, Table 1) received the vaccine 7–10 days prior to rash onset, while wt VZV was detected in 10 patients, confirming that vaccination was in most cases not the source of the illness. Rates of skin rash due to vaccination have been reported to be approximately 5% in healthy children and 10% in adults following varicella vaccination [6, 31]. Only a few studies have applied laboratory

https://doi.org/10.1017/S0950268817001509 Published online by Cambridge University Press

Table 4. *Four SNPs detected for differentiation between wt and vaccine VZV strains*

	SNP				
		ORF62			
VZV Sample	ORF38 69349	106262	107252	108111	GenBank accession number
Dumas-wild	A	T	T	T	X04370
Zostervax-vaccine	G	C	C	C	KY405826-KY405829
Baike-vaccine	G	C	C	C	KY405830-KY405833
Wild:Vaccine (1 : 1) mix	G	C	T	T	Not applicable
ROD – Commercial strain	A	T	T	T	KY405834-KY405837
Case UK-27 (Table 1)	G	C	C	C	Not applicable
Other 43 clinical specimens (Table 1)	A	T	T	T	Not applicable

methods to distinguish between vaccine and wt, 33% (two of six) [32] and 48% of 83 vaccinated subjects (<18 years old) were attributable to the vaccine following varicella vaccination [33]. According to the national guidance [14,15], Zostavax since it is a live vaccine should not be given to patients who have a known primary or acquired immunodeficiency state or patients who are receiving current immunosuppressive therapy, including high-dose corticosteroids, biological therapies or combination therapies. Fourteen of the 27 (51·9%) of the UK patients investigated including four who had received the shingles vaccine (case UK-7, 10, 11 and 13) were immunocompromised or had shingles previously (Table 1). The methodology established would facilitate a broad investigation in countries where VZV vaccination has been launched and contribute to investigations determining whether shingles vaccination can reduce the frequency and severity of clinical symptoms in these patients.

VZV strains have been classified into seven clades including two preliminary ones [6], in addition, clades VIII and IX were reported [12] (Table 3). Genotyping 15 of the 44 strains showed that samples from England were either clade 1 or 3, whilst the samples from China were clade 2 or in once case clade 4 (Table 1) confirming previous reports that clades 1 and 3 predominately circulate in Europe and the Americas, clade 2 is mainly found in Korea, Japan and China, and clades 4 and 5 are mainly detected in India, Nepal, Bangladesh and Africa [6–12, 27, 34]. To show that the technique could be used elsewhere for distinguishing between wt and vaccine VZV in various clades, especially clade 2 as vaccine Oka belongs to clade 2, it is important that this method was shown to work in representative clade 2 samples. It appears that the methodology, which we have established is suitable to support the epidemiological surveillance of VZV during the vaccination era. However, it is still possible that this method may not work for all clade 2 viruses and caution using fixed loci should be taken as there is not only the possibility that recombination episodes may be missed, but that there also may be mixed infections.

The PCR-dependent sequencing approach may randomly amplify the majority of existing virus genomes as we discovered that SNP107252 was detected as vaccine once in one of two samples of case UK-23, who was an unvaccinated HIV positive shingles patient suggesting that VZV genomic mutations may occur in immunocompromised individuals. A wt VZV strain with a characteristic vOka vaccine marker at position 107252 was previously reported, which caused a small outbreak in the USA [35], suggesting that it is essential to differentiate wt VZV from vaccine strains using as a minimum the recommended panel of three vaccine markers within ORF 62 (at positions 106262, 107252 and 108111) and further monitoring the SNPs in larger numbers of patient groups is necessary. The safety and efficacy of varicella vaccination for susceptible individuals and zoster vaccination for immune individuals has been established [36–38]. However, the continual monitoring of patients who have been recently vaccinated is important as potential acquisition in a mixture of vaccine and wt of VZV genomes may occur. The approach established in our study could contribute to such investigations. Deep sequencing directly from clinical specimens using next generation sequencing should identify all variants [39] and recombination, but currently such an approach still remains expensive and may not have the required sensitivity for patients with low viral loads. The methodology established in our

https://doi.org/10.1017/S0950268817001509 Published online by Cambridge University Press

study contributes to VZV surveillance in the era of vaccination.

ACKNOWLEDGEMENTS

We thank Sonia Ribeiro, Gayatri Amirthalingam and Antoaneta Bukasa (Immunisation Department, PHE) for providing the patients' demographic information for the English patients and Pravesh Dhanilall (Virus Reference Department, PHE) for technical support.

DECLARATION OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

1. **Gnann JW Jr., Whitley RJ.** Clinical practice. Herpes zoster. *New England Journal of Medicine* 2002; **347**: 340–346.
2. **Chen SY, *et al.*** Incidence of herpes zoster in patients with altered immune function. *Infection* 2014; **42**: 325–334.
3. **Davison AJ, Scott JE.** The complete DNA sequence of varicella-zoster virus. *Journal of General Virology* 1986; **67**: 1759–1816.
4. **Stow ND, Davison AJ.** Identification of a varicella-zoster virus origin of DNA replication and its activation by herpes simplex virus type 1 gene products. *Journal of General Virology* 1986; **67**: 1613–1623.
5. **Winsome B-M, Richard Nichols R, Breuer J.** Phylogenetic analysis of Varicella-Zoster virus: evidence of intercontinental spread of genotypes and recombination. *Journal of Virology* 2002; **76**: 1971–1979.
6. **Breuer J, *et al.*** A proposal for a common nomenclature for viral clades that form the species varicella-zoster virus: summary of VZV nomenclature meeting 2008, Barts and the London School of Medicine and Dentistry, 24–25 July 2008. *Journal of General Virology* 2010; **91**: 821–828.
7. **Schmidt-Chanasit J, Sauerbrei A.** Evolution and worldwide distribution of varicella-zoster virus clades. *Infection, Genetics and Evolution* 2011; **11**: 1–10.
8. **Kim KH, *et al.*** Genotype of varicella-zoster virus isolates in South Korea. *Journal of Clinical Microbiology* 2011; **49**: 1913–1916.
9. **Liu J, *et al.*** Genotyping of clinical varicella-zoster virus isolates collected in China. *Journal of Clinical Microbiology* 2009; **47**: 1418–1423.
10. **Loparev VN, *et al.*** Global identification of three major genotypes of varicella-zoster virus: longitudinal clustering and strategies for genotyping. *Journal of Virology* 2004; **78**: 8349–8358.
11. **Sergeev N, *et al.*** New mosaic subgenotype of varicella-zoster virus in the USA: VZV detection and genotyping by oligonucleotide-microarray. *Journal of Virology Methods* 2006; **136**: 8–16.
12. **Zell R, *et al.*** Sequencing of 21 varicella-zoster virus genomes reveals two novel genotypes and evidence of recombination. *Journal of Virology* 2012; **86**: 1608–1622.
13. **Takahashi M, *et al.*** Live vaccine used to prevent the spread of varicella in children in hospital. *Lancet* 1974; **304**: 1288–1290.
14. **Public Health England.** Shingles: guidance and vaccination programme (https://www.gov.uk/government/collections/shingles-vaccination-programme).
15. **Department of Health.** Green Book: Chapter 28a Shingles (Herpes zoster) (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/503773/2905109_Green_Book_Chapter_28a_v3_0W.PDF).
16. **Quinlivan ML, *et al.*** Rashes occurring after immunization with a mixture of viruses in the Oka vaccine are derived from single clones of virus. *Journal of Infectious Diseases* 2004; **190**: 793–796.
17. **LaRussa P, *et al.*** Restriction fragment length polymorphism of polymerase chain reaction products from vaccine and wild-type varicellazoster virus isolates. *Journal of Virology* 1992; **66**: 1016–1020.
18. **Hondo R, *et al.*** Distribution of varicella-zoster virus strains carrying a Pst1 site-less mutation in Japan and DNA change responsible for the mutation. *Japanese Journal of Experimental Medicine* 1989; **59**: 233–237.
19. **Harbecke R, *et al.*** A real time PCR assay to identify and discriminate among wild-type and vaccine strains of Varicella Zoster virus and Herpes Simplex virus in clinical specimens, and comparison with the clinical diagnoses. *Journal of Medical Virology* 2009; **81**: 1310–1322.
20. **Higashimoto Y, *et al.*** Discriminating between varicella-zoster virus vaccine and wild-type strains by loop-mediated isothermal amplification. *Journal of Clinical Microbiology* 2008; **46**: 2665–2670.
21. **Ihira M, *et al.*** Cycling probe technology to quantify and discriminate between wild-type varicella-zoster virus and Oka vaccine strains. *Journal of Virology Methods* 2013; **193**: 308–313.
22. **Loparev VN, *et al.*** Improved identification and differentiation of varicella-zoster virus (VZV) wild-type strains and an attenuated varicella vaccine strain using a VZV open reading frame 62-based PCR. *Journal of Clinical Microbiology* 2000; **38**: 3156–3160.
23. **Kaushik KS, *et al.*** Differentiation of wild-type varicella-zoster strains from India and the Oka vaccine strain using a VZV open reading frame-62 based PCR-RFLP technique. *Brazil Journal Infectious Disease* 2008; **12**: 313–315.
24. **Thiele S, *et al.*** Molecular analysis of varicella vaccines and varicella-zoster virus from vaccine-related skin lesions. *Clinical Vaccine Immunology* 2011; **18**: 1058–1066.
25. **Quinlivan ML, *et al.*** Novel genetic variation identified at fixed loci in ORF62 of the Oka varicella vaccine and in a case of vaccine-associated herpes zoster. *Journal of Clinical Microbiology* 2012; **50**: 1533–1538.

26. **Quinlivan M, Breuer J.** Clinical and molecular aspects of the live attenuated Oka varicella vaccine. *Review in Medical Virology* 2014; **24**: 254–273.
27. **Xu S, *et al.*** Nationwide distribution of varicella-zoster virus clades in China. *BMC Infectious Diseases* 2016; **16**: 542.
28. **Quinlivan M, *et al.*** Effect of viral load on the outcome of herpes zoster. *Journal of Clinical Microbiology* 2007; **45**: 3909–3914.
29. **Rozen S, Skaletsky HJ.** Primer3. 1998. Code available at (http://wwwgenome.wi.mit.edu/genome_software/other/primer3.html).
30. **LaRussa P, *et al.*** Restriction fragment length polymorphism of polymerase chain reaction products from vaccine and wild-type varicella-zoster virus isolates. *Journal of Virology* 1992; **66**: 1016–1020.
31. **Ampofo K, *et al.*** Persistence of immunity to live attenuated varicella vaccine in healthy adults. *Clinical Infectious Diseases* 2002; **34**: 774–779.
32. **Chun C, *et al.*** Laboratory characteristics of suspected herpes zoster in vaccinated children. *Pediatric Infectious Disease Journal* 2011; **30**: 719–721.
33. **Weinmann S, *et al.*** Incidence and clinical characteristics of herpes zoster among children in the varicella vaccine era, 2005–2009. *Journal of Infectious Diseases* 2013; **208**: 1859–1868.
34. **Peters GA, *et al.*** A full-genome phylogenetic analysis of varicella-zoster virus reveals a novel origin of replication-based genotyping scheme and evidence of recombination between major circulating clades. *Journal of Virology* 2006; **80**: 9850–9860.
35. **Lopez AS, *et al.*** Transmission of a newly characterized strain of varicella-zoster virus from a patient with herpes zoster in a long-term-care facility, West Virginia, 2004. *Journal of Infectious Diseases* 2008; **197**: 646–653.
36. **Kenneth E, *et al.*** Efficacy, safety, and tolerability of herpes zoster vaccine in persons aged 50–59 years. *Clinical Infectious Diseases* 2012; **54**: 922–928.
37. **Marietta V, *et al.*** The effectiveness of the varicella vaccine in clinical practice. *New England Journal of Medicine* 2001; **344**: 955–960.
38. **LaRussa P, *et al.*** Viral strain identification in varicella vaccines with disseminated rashes. *Pediatric Infectious Disease Journal* 2000; **19**: 1037–1039.
39. **Depledge DP, *et al.*** Viral genome sequencing proves nosocomial transmission of fatal varicella. *Journal of Infectious Diseases* 2016; **214**: 1399–1402.