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(54) Titre : PROTEINE VP26 IMMUNOREACTIVE DU VIRUS VARICELLE-ZONA (VZV) ET SON UTILISATION
DIAGNOSTIQUE

(54) Title: VARICELLA ZOSTER VIRUS (VZV) IMMUNOREACTIVE PROTEIN VP26 AND ITS DIAGNOSTIC USE

(57) **Abrégé/Abstract:**

The present invention relates to immunoreactive peptides which are homologous with the region of amino acid positions 12 to 235 of the varicella zoster virus protein VP26, to nucleic acids which encode these peptides and to the use of the peptides or nucleic acids for diagnosing an infection with varicella zoster virus.



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Abstract of the disclosure:

**Varicella zoster virus (VZV) immunoreactive protein VP26
and its diagnostic use**

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**Varicella zoster virus (VZV) immunoreactive protein VP26
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The present invention relates to immunoreactive peptides
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protein VP26, to nucleic acids which encode these
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for diagnosing an infection with varicella zoster virus.

10

In accordance with the classification of the Inter-
national Committee on Taxonomy of Viruses (ICTV), VZV is
assigned to the Herpesviridae family. In 75% of cases,
the primary infection takes place not later than the age
15 of 15 and usually takes an inapparent course. By
contrast, infection of adults who have not previously
had any contact with the virus and in persons who are
naturally or therapeutically immunosuppressed can be
associated with severe symptoms. Infection of the fetus
20 also leads to severe symptoms since the virus is able to
cross the placenta, and maternal antibodies afford no
protection at this time. Following the primary
infection, the virus persists throughout life in sensory
ganglia. After reactivation, the VZV spreads over the
25 peripheral nerves in sensory ganglia and then gives rise
to herpes zoster.

Seventy open reading frames (ORF), including the open
reading frames for the currently known glycoproteins gpI
30 (ORF 68), gpII (ORF 31), gpIII (ORF 37), gpIV (ORF 67),
gpV (ORF 14) and gpVI (ORF 60), can be deduced from the
sequence of the VZV genome, which has been completely
elucidated and has a length of 124,884 bp (Dumas strain;
(A.J. Davison & J.E. Scott (1986), J. Gen. Virol. 67,
35 1759-1816)). In each case, the amino acid sequence
deduced from the nucleotide sequence displays differing

degrees of homology with glycoproteins gE, gB, gH, gI, gC and gL of herpes simplex virus (HSV). However, there is nothing to suggest that the sequence homology can also imply a function homology. The open reading frames
5 of glycoproteins gpI, gpII, gpIII and gpV have been confirmed by means of molecular biology.

Only a few immunogenic VZV capsid proteins have been identified and characterized. D.R. Harper and C. Grose
10 ((1989), J. Infect. Dis. 159, 444-451) describe the immunoreactivity of the p32/p36 complex, which is assigned to the nucleocapsid. In addition, A. Vafai et al. ((1990), Virus Res. 15, 163-174) describe the cross reactivity of human VZV-reactive sera, which recognize
15 both the varicella zoster virus nucleocapsid protein and the HSV nucleocapsid protein. None of the authors proposes approaches which would make it possible to use these antigens in diagnostic test methods.

20 There are a very large number of different serological methods for checking the level of VZV-specific immunity, with these methods ranging from radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), fluorescent-antibody membrane antigen assay (FAMA) and the immuno-
25 fluorescence test (IFT) to complement fixation (CF). Most of these methods detect VZV-specific antibodies. Virus material which has been isolated following elaborate cultivation on human fibroblast cultures, and has been prepared for diagnostic use in accordance with
30 special methods, is frequently employed as the antigen.

The isolation of VZV antigens from infected fibroblasts is associated with the risk of infecting the personnel who are entrusted with this task. In addition, the
35 preparation is very costly and time-consuming, inter alia because the virus is not released from the infected cells, and special purification methods are therefore required. When the antigen is to be used without prior purification for an immunochemical test, the VZV-

infected cells are disrupted by sonication, and the antigen is employed directly after dilution for coating microtitration plates, for example. Since, in this method, it is mainly cell-specific antigens which are
5 bound to the microtitration plate wells in addition to the virus-specific antigens, the cell-specific antigens can, particularly in association with certain diseases, for example autoimmune diseases, give rise to false-positive results and consequently to erroneous
10 diagnoses. Other test methods which are based on purified viral glycoproteins, for example a glycoprotein ELISA, require purification methods which, to a marked degree, are more elaborate and involve heavier losses, so that it has so far scarcely been possible to
15 establish immunochemical diagnostic tests on a relatively large scale. Cross reactivities with HSV-specific antibodies, and, as a consequence, false-positive results, are also frequently to be observed in the case of the glycoprotein ELISA due to the pronounced
20 homology which exists between the glycoproteins of the α -herpesviruses.

A possible solution to the above-cited problems lies in the use of recombinant proteins, which can be prepared
25 in large quantity in a heterologous system, e.g. in *Escherichia coli* (*E. coli*). There is no possibility of infecting personnel with VZV in this system. It also provides the possibility of carrying out a differential diagnosis which is directed towards specific viral
30 proteins. In particular, the reactive region of the VZV proteins can be delimited to such an extent that cross reactivities with antibodies which are specific for other herpesviruses can be virtually or completely ruled out. The proteins which meet these requirements can be
35 expressed as hybrid proteins, with either a host-specific protein, for example the *E. coli* maltose-binding protein (MBP) or an N-terminally located sequence of 6 histidine residues (His tag), contributing, as the fusion partner, to the stability of

the expression product. These hybrid proteins can then, by way of single-step affinity purifications, be used directly, in almost pure, and consequently contamination-free form, for coating diagnostically
5 utilizable surfaces, for example ELISA microtitration plates. Proteins which meet the abovementioned criteria have not so far been described for the VZV system.

Surprisingly, it was found to be possible to stably
10 express a part of VZV ORF23, as VP26*, in E. coli. It was furthermore found, surprisingly, that VP26* is immuno-reactive and can be employed advantageously in diagnosis.

15 The present invention consequently relates to an immuno-reactive peptide which is homologous with the AA 12 to 235 region of VZV VP26 or which essentially contains the amino acids 12 to 235 region of VP26. Those immuno-reactive peptides which display naturally varying amino
20 acid sequences due to VZV strain variations are to be expressly included in this context.

The invention furthermore relates to nucleic acids, i.e. DNA or RNA, which encode the abovementioned immuno-
25 reactive peptides according to the invention. This is, in particular, the nucleic acid which has the sequence depicted in Table 1. However, in addition to this, nucleic acids which hybridize with the abovementioned nucleic acids under stringent conditions and encode
30 peptides which are recognized by antibodies which are directed against VZV but not by antibodies which are directed against other herpesviruses are also to be included.

35 The invention also relates to immunoreactive peptides which can be prepared by expressing the nucleic acid having the sequence depicted in Table 1 or one of the abovementioned nucleic acids which hybridize under stringent conditions, wherein all the peptides are

recognized by antibodies which are directed against VZV but not by antibodies which are directed against other herpesviruses.

5 The invention furthermore relates to an immunochemical method which makes it possible to detect antibodies against VZV. In this method, one or more of the said immunoreactive peptides is/are brought into contact with a sample, for example a blood, plasma or serum sample
10 from a patient, which is to be investigated for the presence of VZV-specific antibodies. Methods with which the skilled person is familiar are then used to establish wherever antibodies from the sample bind to the immunoreactive peptides employed or enter into
15 another immunological interrelationship, for example a competition, with these peptides.

The present invention also relates to the use of the abovementioned nucleic acid according to the invention
20 for detecting VZV by means of nucleic acid hybridization.

In addition to this, the present invention relates to a test kit for detecting antibodies against VZV, which kit
25 comprises an immunoreactive peptide according to the invention, and to a test kit for detecting VZV, which kit comprises the nucleic acid according to the invention.

30 Detailed description of the invention:

In order to locate diagnostically utilizable immuno-reactive regions, immunoreactive proteins of VZV were identified by immunoscreening a VZV genomic library. For
35 this, a VZV genomic library was constructed as a phage library in the Zap Express System (Stratagene). This library was then used to carry out an immunoscreening employing a serum pool composed of 25 VZV-reactive human serum. The DNA of clones which were assessed as being

positive were converted into the circular form, sequenced and assigned to the corresponding region of the VZV sequence. With the aid of this method, it was possible to isolate an immunoreactive clone which
5 expressed AA12-235 of ORF23. No expression product for this reading frame had previously been reported in the VZV system. However, it is known from studies of the homology between VZV and HSV that ORF23 corresponds to HSV1 UL36 (A.J. Davison & J.E. Scott. (1986), J. Gen.
10 Virol. 67, 1759-1816). However, at 116 AA, the corresponding herpes simplex virus gene product is substantially shorter and does not exhibit any marked homology either. It had not previously been possible to demonstrate any immunoreactivity (of human sera) to either
15 the gene product of VZV ORF23 or the gene product of HSV UL36. On the basis of the above results, the ORF23 gene product (VP26) constitutes a possible candidate for a diagnostic test method. As a consequence, both the entire ORF23 and also the region which was truncated N-
20 terminally were subcloned into the vector pMAL-c2. This resulted in the constructs pMAL-VP26 and pMAL-VP26*. These were sequenced in an overlapping, bidirectional manner. The sequence does not display any differences as compared with the published sequence (A.J. Davison &
25 J.E. Scott. (1986), J. Gen. Virol. 67, 1759-1816). Expression of these constructs showed that while it was possible to express the truncated protein stably this was not the case with the whole protein. The immunoreactivity of the pMAL-VP26* product was found to
30 be positive using the serum pool (see above). The immunoreactivity was also confirmed after the VZV-specific DNA fragment had been subcloned from vector pMAL-VP26* into the vector pQE30 and expressed in the pQE system. It was therefore possible to rule out any
35 possible false-positive assessment arising from the MBP fusion moiety.

In an ELISA assessment, pMAL-VP26* was found, with a calculated cut off = 187 (average value of the negative

sera + 3 times the standard deviation) to give a sensitivity of 69% (n=13) and a specificity of 98% (n=41) or, with a freely selected cut off of 120, a sensitivity of 85% and a specificity of 95%, for IgM diagnosis. When
5 pQE-VP26* is used, the sensitivity with the calculated cut off = 173 (average value of the negative sera + 3 times the standard deviation) is 62% (n=13) with the specificity being 100% (n=41) or, with a freely selected cut off of 150, the sensitivity is 77% and the
10 specificity is 95%.

In an IgG ELISA assessment, pMAL-VP26* was found, with a calculated cut off at 210 (average value of the negative sera + 3 times the standard deviation), to give a
15 sensitivity of 35% (n=49) and a specificity of 100% (n=5) or, with a freely selected cut off at 100, a sensitivity of 59% and a specificity of 80%, for IgG diagnosis. When pQE-VP26* is used, the sensitivity with the calculated cut off of 215 (average value of the
20 negative sera + 3 times the standard deviation) is 53% with the specificity being 100%, or, with the freely selected cut off at 130, the sensitivity is 82% and the specificity is 80%.

25 **Sequence 1:** Amino acid residues 1-235 of VP26 (ORF23) (Ellen strain). 235 AA in all; theoretical molecular weight 24.4 kDa. The region in bold corresponds to the AA 12 - 235 VP26* immunoreactive fragment having a total of 224 AA and a theoretical molecular weight of 23 kDa.
30 The rhombus (#) symbolizes the stop codon. The numbering of the amino acids begins with the methionine (start codon) of the published sequence for ORF23 (A.J. Davison & J.E. Scott. (1986), J. Gen. Virol. 67, 1759-1816):

Met Thr Gln Pro Ala Ser Ser Arg Val Val Phe Asp Pro Ser Asn Pro
Thr Thr Phe Ser Val Glu Ala Ile Ala Ala Tyr Thr Pro Val Ala Leu
Ile Arg Leu Leu Asn Ala Ser Gly Pro Leu Gln Pro Gly His Arg Val
Asp Ile Ala Asp Ala Arg Ser Ile Tyr Thr Val Gly Ala Ala Ala Ser
Ala Ala Arg Ala Arg Ala Asn His Asn Ala Asn Thr Ile Arg Arg Thr
Ala Met Phe Ala Glu Thr Asp Pro Met Thr Trp Leu Arg Pro Thr Val
Gly Leu Lys Arg Thr Phe Asn Pro Arg Ile Ile Arg Pro Gln Pro Pro
Asn Pro Ser Met Ser Leu Gly Ile Ser Gly Pro Thr Ile Leu Pro Gln
Lys Thr Gln Ser Ala Asp Gln Ser Ala Leu Gln Gln Pro Ala Ala Leu
Ala Phe Ser Gly Ser Ser Pro Gln His Pro Pro Pro Gln Thr Thr Ser
Ala Ser Val Gly Gln Gln Gln His Val Val Ser Gly Ser Ser Gly Gln
Gln Pro Gln Gln Gly Ala Gln Ser Ser Thr Val Gln Pro Thr Thr Gly
Ser Pro Pro Ala Ala Gln Gly Val Pro Gln Ser Thr Pro Pro Pro Thr
Gln Asn Thr Pro Gln Gly Gly Lys Gly Gln Thr Leu Ser His Thr Gly
Gln Ser Gly Asn Ala Ser Arg Ser Arg Arg Val #

The corresponding nucleotide sequence is depicted in
Table 1.

Table 1:

	ATGACACAACCCGCATCGTCTCGTGTAAGTCCTTTGATCCCAGCAACCCACCACATTTCG	
1	-----+-----+-----+-----+-----+-----+	60
	TACTGTGTTGGGCGTAGCAGAGCACATCAGAACTAGCGTCGTTGGGGTGGTGTAAAAGC	
	MetThrGlnProAlaSerSerArgValValPheAspProSerAsnProThrThrPheSer	
	M' T Q P A S S R V V F D P S N P T T P S	
	GTGGAAGCAATTGCCGCTTACACCCCCGTTGCTTTAATACGACTTTTAAACGCCAGTGGA	
61	-----+-----+-----+-----+-----+-----+	120
	CACCTTCGTTAACCCGAATGTGGGGGCAACGAAATTATGCTGAAAATTTGCGGTCACCT	
	ValGluAlaIleAlaAlaTyrThrProValAlaLeuIleArgLeuLeuAsnAlaSerGly	
	V E A I A A Y T P V A L I R L L N A S G	
	CCTTTGCAACCTGGTCACCGTGTGGACATCGCTGATGCCAGAAGCATTTACACCGTGGA	
121	-----+-----+-----+-----+-----+-----+	180
	GGAAACGTTGGACCAGTGGCACACCTGTAGCGACTACGGTCTTCGTAAATGTGGCACCCCT	
	ProLeuGlnProGlyHisArgValAspIleAlaAspAlaArgSerIleTyrThrValGly	
	P L Q P G H R V D I A D A R S I Y T V G	
	GCCGCGGCCAGTGCCGCGCGTGACGCGCTAACCATAATGCAAATACGATACGCCGAACG	
181	-----+-----+-----+-----+-----+-----+	240
	CGGCGCCCGGTCACGGCGCGCACGTGCGCGATTGGTATTACGTTTTATGCTATGCGGCTTGC	
	AlaAlaAlaSerAlaAlaArgAlaArgAlaAsnHisAsnAlaAsnThrIleArgArgThr	
	A A A S A A R A R A N H N A N T I R R T	
	GCCATGTTTGGCGAGACTGACCCCTATGACATGGTTAAGACCAACGGTTGGCTTAAAACGT	
241	-----+-----+-----+-----+-----+-----+	300
	CGGTACAAACGGCTCTGACTCGGATACTGTACCAATTCTGOTTGCCAACCGAATTTTGCA	
	AlaMetPheAlaGluThrAspProMetThrTrpLeuArgProThrValGlyLeuLysArg	
	A M F A E T D P M T W L R P T V G L K R	

ACGTTTAACCCGCGTATTATACGACCACAACCCCCAAATCCATCCATGAGTTTGGAATC
301 -----+-----+-----+-----+-----+-----+ 360
TGCAAATTGGGCGCATAATATGCTGGTGTGGGGGTTAGGTAGGTACTCAAACCCTTAG
ThrPheAsnProArgIleIleArgProGlnProProAsnProSerMetSerLeuGlyIle
T P N P R I I R P Q P P N P S M S L G I

TCGGGGCCTACTATATTGCCGCAAAAAACACAGAGCGCCGATCAGTCTGCTTTACAACAG
361 -----+-----+-----+-----+-----+-----+ 420
AGCCCCGGATGATATAACGGCGTTTTTTGTGTCTCGCGGCTAGTCAGACGAAATGTTGTC
SerGlyProThrIleLeuProGlnLysThrGlnSerAlaAspGlnSerAlaLeuGlnGln
S G P T I L P Q K T Q S A D Q S A L Q Q

CCCGCCGCGTTGGCGTTTTCGGGATCATCCCCGCAACACCCCCCACCTCAAACGACGTCG
421 -----+-----+-----+-----+-----+-----+ 480
GGGCGGCGCAACCGCAAAAGCCCTAGTAGGGGCGTTGTGGGGGTGGAGTTTGCTGCAGC
ProAlaAlaLeuAlaPheSerGlySerSerProGlnHisProProProGlnThrThrSer
P A A L A F S G S S P Q H P P P Q T T S

GCATCCGTTGGACAACAGCAACACGTGGTGTGGGGTCTTCTGGACAACAACCGCAACAG
481 -----+-----+-----+-----+-----+-----+ 540
CGTAGGCAACCTGTTGTGCTGTGTCACCACAGCCCCAGAAGACCTGTTGTTGGCGTTGTC
AlaSerValGlyGlnGlnGlnHisValValSerGlySerSerGlyGlnGlnProGlnGln
A S V G Q Q Q H V V S G S S G Q Q P Q Q

GGAACACAGTCAAGCACTGTCCAGCCAACAACCGGATCACCGCCCGGGCCCAAGGCGTG
541 -----+-----+-----+-----+-----+-----+ 600
CCTCGTGTGAGTTGCTGACAGGTCCGTTGTTGGCCTAGTGGCGGGCGCGGGTTCCGCAC
GlyAlaGlnSerSerThrValGlnProThrThrGlySerProProAlaAlaGlnGlyVal
G A Q S S T V Q P T T G S P P A A Q G V

CCACAGTCTACCCCGCCCCCAACCCAAAATACCCCCCAGGGGGGTAAAGGGACAGACCTTG
601 -----+-----+-----+-----+-----+-----+-----+ 660
GGTGTTCAGATGGGGCGGGGGTTGGGTTTATGGGGGGTCCCCCATTCCCTGTCTGGAAC
ProGlnSerThrProProProThrGlnAsnThrProGlnGlyGlyLysGlyGlnThrLeu
P Q S T P P P T Q N T P Q G G K G Q T L

TCACACACGGGACAATCTGGAAACGCTTCAAGAAGTCGTAGGGTG
661 -----+-----+-----+-----+-----+-----+-----+ 705
AGTGTGTGCCCTGTAGACCTTTGCGAAGTTCTTCAGCATCCCAC
SerHisThrGlyGlnSerGlyAsnAlaSerArgSerArgArgVal
S H T G Q S G N A S R S R R V

Examples

Example 1:

Isolation of viral VZV virions and DNA extraction:

5

Sonication was used to release the virions from VZV-infected fibroblasts. The viruses were purified from cell constituents through a linear sucrose gradient (20-70% (w/v)). Following incubation with DNase I, the viral
10 DNA was released from the virions by treating them with proteinase K and sodium dodecyl sulfate (SDS). After having been extracted with phenol/chloroform, the viral DNA was precipitated with ethanol.

15 **Example 2:**

Construction of a genomic expression library and immuno-screening:

The VZV genomic DNA (see Example 1) was partially
20 digested by incubating with the restriction enzyme SauIIIA and cloned into the BamHI cleavage site of lambda DNA of the ZAP Express vector system (Stratagene) and packaged in lambda phages. Following infection of *E. coli* (XL1-Blue MRF), the phage titer was found to be $5 \times$
25 10^5 /ml. By means of blue/white screening it was established that 25% of these phages did not harbor any inserted DNA fragments. XL1-Blue cells were infected with the phages, transferred to LB top agar and incubated at 42°C for 3 hours on LB agar plates. After
30 laying isopropyl- β -D-thiogalactopyranoside-soaked nitrocellulose membranes on them, the LB plates were incubated at 37°C for 6-10 hours. The membranes were then analyzed for immunoreactive clones by means of immunostaining using a serum pool composed of 25 VZV-
35 reactive human sera as the first antibody and a peroxidase-coupled rabbit anti-human IgG as the second antibody. Immunoreactive clones were plaque-purified. The region encoding the phagemid vector pBK/CMV,

including the inserted VZV DNA, was excised using the ExAssist helper phage system (Stratagene) and circularized. The VZV DNA moiety was analyzed by sequencing and identified by database searching (GCG Blastn program).

Example 3:

Cloning ORF23 (VP26):

10 The viral DNA (see Example 1) or the phagemid vector, pBK/CMV-23, obtained from the immunoreactive lambda phage served as a template for amplifying the ORF23 (VP26) or, respectively, the ORF23 fragment (VP26*) which was inserted in vector pBK/CMV-23. The following primers
15 were used as amplification oligonucleotides:

VP26: VP26H 5' GAATTCCGGATGACACAACCCGCATCGTCTCGTGTA 3';
 VP26R 5' GCTCTAGATTACACCCTACGACTTCTTGAAGCGTTTCC 3'.
VP26*: VP26*H 5' GGAATTCCGCGCCTGCAGGTCGACACTAGTGGAT 3'
 VP26*R 5' GCTCTAGATTACACCCTACGACTTCTTGAAGCGTTTCC 3'.

These oligonucleotides are complementary to the corresponding segment of the published VZV sequence (A.J. Davison & J.E. Scott. (1986), J. Gen. Virol. 67, 1759-1816) or the sequence of vector pBK/CMV (Stratagene), but they contain, at their 5' termini, a restriction cleavage site sequence which did not hybridize the template DNA. After amplification had taken place, the
25 amplificate, of 726 bp or 714 bp in size, respectively, was cleaved terminally with the restriction enzymes EcoRI and XbaI and ligated into expression vector pMAL-

c2, which had been linearized previously with EcoRI and XbaI. The entire ORF23 was completely sequenced in an overlapping, bidirectional manner. The vectors were designated pMAL-VP26 and pMAL-VP26*, respectively.

5 In addition, the region (VP26*) encoding the immunoreactive protein was cloned into vector pQE30. VZV genomic DNA was used as the template DNA. The following primers were used as amplification oligonucleotides:

10 VP26*: VP26* 5' CGGATCCGATCCCAGCAACCCACAC 3';
VP26R 5' GCTCTAGATTACACCCTACGACTTCTTGAAGCGTTTCC 3'.

These oligonucleotides are complementary to the corresponding segment of the published VZV sequence (A.J. Davison & J.E. Scott. (1986), J. Gen. Virol. 67, 1759-1816), but they contain, at their 5' termini, a restriction cleavage site sequence which did not hybridize with the template DNA. After amplification had taken place, the amplificate, which was 690 bp in size,
20 was cleaved terminally with the restriction enzyme EcoRI and ligated into expression vector pQE30, which had been linearized previously with EcoRI and SmaI. The vector was designated pQE-VP26*.

25 **Example 4**

a) Preparation of the recombinant proteins pMal-VP26* and pQE-VP26*:

Expression and purification of the recombinant protein pMAL-VP26* was carried out by means of affinity chromatography in accordance with the manufacturer's instructions (New England Biolabs, 800-21S).

Expression and purification of recombinant protein pQE-VP26* was carried out by means of metal affinity chromatography under native conditions in accordance with the manufacturer's instructions (Clontech, Talon Metal Affinity Resin, PT1320-1).

b) Preparation of pMal-VP26* or pQE-VP26* solid phase I (novel system):

Type B microtitration plates (from Greiner) were
5 incubated, at 4°C for 18 hours, with 110 µl per well of
coating solution (1 µg of recombinant pMal-VP26*/ml or
1 µg of recombinant pQE-VP26*/ml in 50 mM sodium
carbonate buffer, pH 9.5). The wells of the micro-
titration plates were then washed three times with 300
10 µl of washing solution on each occasion (50 mM
Tris/EDTA, pH 7.0-7.4 + 0.5% BSA).

For testing in an anti-VZV IgG enzyme immunoassay, the
recombinant protein pQE-VP26* was coated under the same
conditions as described above, but using a concentration
15 of 2 µg/ml.

c) Enzyme immunoassay for detecting anti-VZV IgM anti-
bodies:

20 The enzyme immunoassay for detecting anti-VZV IgM anti-
bodies was carried out as follows:

The sera were initially diluted 1:84 in POD sample
buffer (Behring Diagnostics, OWBE 945) and then
preincubated at room temperature for 15 minutes with RF
25 adsorbent (Behring Diagnostics, OUCG 945) in a ratio of
1:2. In each case, 100 µl of the sera which had been
prediluted as described above were incubated, at 37°C
for 1 h, in the wells of the microtitration plates which
had been prepared as described in Example 4b. After the
30 plates had been washed 3 times with POD wash solution
(Behring Diagnostics, OSEW 965), 100 µl of the anti-
human IgM/POD conjugate (Behring Diagnostics, batch
4241313), diluted in Microbiol conjugate buffer (Behring
Diagnostics, OUWW 935) in a ratio of 1:25 in the case of
35 pQE-VP26* and 1:50 in the case of pMal-VP26*, were
pipetted in. The incubation of 1 hour (at +37°C) was
terminated by 3 further washing steps. The bound
peroxidase activity, which correlates directly with the

number of bound VZV antibody molecules, was determined by adding H_2O_2 /tetramethylbenzidine (Behring Diagnostics, OUVG 945). After 30 minutes at room temperature, substrate conversion was stopped by adding 0.5 M
5 sulfuric acid (Behring Diagnostics, OSFA 965) and the extinction was measured at 450 nm.

Anti-VZV-positive and anti-VZV-negative sera were investigated both in the Enzygnost anti-VZV/IgM reference system (Behring Diagnostics, OWLW 155) and in
10 the novel enzyme immunoassay.

The results (extinction units) of the investigation are given in Table 2.

15 In an ELISA assessment, pMAL-VP26* was found, with a calculated cut off = 187 (average value of the negative sera + 3 times the standard deviation) to give a sensitivity of 69% (n=13) and a specificity of 98% (n=41) or, with a freely selected cut off of 120, a
20 sensitivity of 85% and a specificity of 95% for IgM diagnosis. When pQE-VP26* is used, the sensitivity with the calculated cut off = 173 (average value of the negative sera + 3 times the standard deviation) is 62% (n=13) and the specificity is 100% (n=41) or, with a
25 freely selected cut off of 150, the sensitivity is 77% and the specificity is 95%.

Example 5

a) Enzyme immunoassay for detecting anti-VZV IgG anti-
30 bodies:

In order to detect anti-VZV IgG antibodies in the enzyme immunoassay, 100 μ l of the sera which had been prediluted 1:100 in POD sample buffer (Behring
35 Diagnostics, OWBE 945) were in each case pipetted into the microtiter plate which had been prepared as described in Example 4b and incubated at 37°C for 1 h.

After the plate had been washed 3 times with POD wash solution (Behring Diagnostics, OSEW 965), 100 μ l of

anti-human IgG/POD conjugate (Behring Diagnostics batch 243713), diluted 1:50 in Microbiol conjugate buffer (Behring Diagnostics, OUWW 935), were pipetted in. The 1-hour incubation (at +37°C) was terminated with a
5 further three washing steps. The bound peroxidase activity, which correlates directly with the number of bound VZV antibody molecules, was determined by adding H₂O₂/tetramethylbenzidine (Behring Diagnostics, OUVG 945). After 30 minutes at room temperature, substrate
10 conversion was stopped by adding 0.5 M sulfuric acid (Behring Diagnostics, OSFA 965) and the extinction was measured at 450 nm.

Anti-VZV-positive and anti-VZV-negative sera were
15 investigated both in the Enzygnost anti-VZV/IgG reference system (Behring Diagnostics, OWLT 155) and in the novel enzyme immunoassay. The results (extinction units) of the investigation are given in Table 3.

20 In an ELISA assessment, pMAL-VP26* was found, with a calculated cut off of 210 (average value of the negative sera + 3 times the standard deviation) to give a sensitivity of 35% (n=49) and a specificity of 100% (n=5) or, with a freely selected cut off of 100, a sensitivity of
25 59% and a specificity of 80%, for IgG diagnosis. When pQE-VP26* is used, the sensitivity with the calculated cut off of 215 (average value of the negative sera + 3 times the standard deviation) is 53% and the specificity is 100%, or, with the freely selected cut off at 130,
30 the sensitivity is 82% and the specificity is 80%.

Table 2:

No.	Status	ELISA IgM Reference	ELISA IgM pMal- VP26* 1 µg/ml	ELISA IgM pQE- VP26* 1 µg/ml	No.	Status	ELISA IgM Reference	ELISA IgM pMal- VP26* 1 µg/ml	ELISA IgM pQE- VP26* 1 µg/ml
		Conj. 1:50	Conj. 1:50	Conj. 1:25			Conj. 1:50	Conj. 1:50	Conj. 1:25
		Serum 1:42	Serum 1:168	Serum 1:168			Serum 1:42	Serum 1:168	Serum 1:168
1	-	11	86	62	29	-	16	19	104
2	-	22	38	100	30	-	8	13	44
3	-	16	31	72	31	-	3	23	47
4	-	57	23	88	32	-	50	23	101
5	+	133	399	88	33	-	9	32	117
6	-	0	161	118	34	+	250	187	204
7	-	10	28	77	35	+	291	1079	470
8	-	47	45	60	36	-	26	52	44
9	-	7	16	63	37	+	139	66	123
10	+	132	173	160	38	-	38	87	49
11	-	71	117	70	39	-	26	25	40
12	-	0	32	75	40	-	49	13	63
13	-	17	75	66	41	-	5	20	34
14	-	11	16	57	42	-	27	43	80
15	-	23	27	73	43	-	51	49	161
16	-	11	27	59	44	-	8	38	100
17	+	979	1720	532	45	-	21	38	133
18	-	9	18	44	46	-	10	33	82
19	-	34	30	71	47	-	29	18	35
20	+	293	630	215	48	-	30	66	97
21	+	126	139	259	49	-	21	35	97
22	-	61	277	169	50	-	56	64	82
23	+	292	808	154	51	-	5	40	56
24	+	581	98	115	52	-	97	55	37
25	+	321	821	304	53	-	2	19	35
26	+	509	519	375	54	-	31	21	39
27	+	298	>2500	694					
28	-	29	36	29					

Table 3:

No.	Status	ELISA IgG Reference	ELISA IgG pMal VP26* 1 µg/ml	ELISA IgG pQE VP26* 2 µg/ml	No.	Status	ELISA IgG Reference	ELISA IgG pMal VP26* 1 µg/ml	ELISA IgG pQE VP26* 2 µg/ml
		Conj. 1:50	Conj. 1:50	Conj. 1:50			Conj. 1:50	Conj. 1:50	Conj. 1:50
		Serum 1:231	Serum 1:100	Serum 1:100			Serum 1:231	Serum 1:100	Serum 1:100
1	+	1014	176	137	28	+	549	89	172
2	+	302	87	356	29	-	34	87	120
3	+	642	97	314	30	-	76	55	128
4	+	612	87	0	31	-	0	38	101
5	+	1383	181	215	32	-	41	94	116
6	+	930	132	118	33	-	8	147	177
7	+	653	39	145	34	+	1315	860	340
8	+	915	47	469	35	+	1629	282	187
9	+	570	23	220	36	+	469	218	287
10	+	1770	131	253	37	+	693	181	346
11	+	990	104	76	38	+	1449	296	169
12	+	698	227	335	39	+	1139	85	193
13	+	141	62	501	40	+	1509	64	118
14	+	338	39	44	41	+	457	110	159
15	+	556	113	246	42	+	343	304	545
16	+	791	45	423	43	+	1455	511	244
17	+	2982	264	258	44	+	1030	174	245
18	+	1205	192	295	45	+	528	50	184
19	+	694	80	100	46	+	510	161	353
20	+	1143	270	340	47	+	300	94	290
21	+	1405	377	256	48	+	490	91	157
22	+	539	42	72	49	+	888	348	277
23	+	2449	533	530	50	+	1301	83	195
24	+	1923	1117	620	51	+	704	247	139
25	+	1420	95	54	52	+	2523	2325	757
26	Primary +	1194	277	66	53	+	447	99	153
27	Zoster +	2087	341	204	54	+	848	149	167

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: Dade Behring Marburg GmbH
- (B) STREET: Emil-von-Behring Str. 76
- (C) CITY: Marburg
- (E) COUNTRY: Germany
- (F) POSTAL CODE (ZIP): 35001 Marburg
- (G) TELEPHONE: 06421/39-2332
- (H) TELEFAX: 06421/39-3631

(ii) TITLE OF INVENTION: Varicella zoster virus (VZV) immunoreactive protein VP26 and its diagnostic use

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: BERESKIN & PARR
- (B) STREET: 40 King Street West
- (C) CITY: Toronto
- (D) STATE: Ontario
- (E) COUNTRY: Canada
- (F) ZIP: M5H 3Y2

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: 2,255,106
- (B) FILING DATE: December 16, 1998
- (C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Gravelle, Micheline
- (B) REGISTRATION NUMBER: 4189
- (C) REFERENCE/DOCKET NUMBER: 9694-172

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: (416) 364-7311
- (B) TELEFAX: (416) 361-1398

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 235 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Varicella-Zoster-Virus
- (B) STRAIN: Ellen

(vii) IMMEDIATE SOURCE:

- (B) CLONE: VP26

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(viii) POSITION IN GENOME:
 (B) MAP POSITION: ORF23

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Met	Thr	Gln	Pro	Ala	Ser	Ser	Arg	Val	Val	Phe	Asp	Pro	Ser	Asn	Pro	1	5	10	15
Thr	Thr	Phe	Ser	Val	Glu	Ala	Ile	Ala	Ala	Tyr	Thr	Pro	Val	Ala	Leu	20	25	30	
Ile	Arg	Leu	Leu	Asn	Ala	Ser	Gly	Pro	Leu	Gln	Pro	Gly	His	Arg	Val	35	40	45	
Asp	Ile	Ala	Asp	Ala	Arg	Ser	Ile	Tyr	Thr	Val	Gly	Ala	Ala	Ala	Ser	50	55	60	
Ala	Ala	Arg	Ala	Arg	Ala	Asn	His	Asn	Ala	Asn	Thr	Ile	Arg	Arg	Thr	65	70	75	80
Ala	Met	Phe	Ala	Glu	Thr	Asp	Pro	Met	Thr	Trp	Leu	Arg	Pro	Thr	Val	85	90	95	
Gly	Leu	Lys	Arg	Thr	Phe	Asn	Pro	Arg	Ile	Ile	Arg	Pro	Gln	Pro	Pro	100	105	110	
Asn	Pro	Ser	Met	Ser	Leu	Gly	Ile	Ser	Gly	Pro	Thr	Ile	Leu	Pro	Gln	115	120	125	
Lys	Thr	Gln	Ser	Ala	Asp	Gln	Ser	Ala	Leu	Gln	Gln	Pro	Ala	Ala	Leu	130	135	140	
Ala	Phe	Ser	Gly	Ser	Ser	Pro	Gln	His	Pro	Pro	Pro	Gln	Thr	Thr	Ser	145	150	155	160
Ala	Ser	Val	Gly	Gln	Gln	Gln	His	Val	Val	Ser	Gly	Ser	Ser	Gly	Gln	165	170	175	
Gln	Pro	Gln	Gln	Gly	Ala	Gln	Ser	Ser	Thr	Val	Gln	Pro	Thr	Thr	Gly	180	185	190	
Ser	Pro	Pro	Ala	Ala	Gln	Gly	Val	Pro	Gln	Ser	Thr	Pro	Pro	Pro	Thr	195	200	205	
Gln	Asn	Thr	Pro	Gln	Gly	Gly	Lys	Gly	Gln	Thr	Leu	Ser	His	Thr	Gly	210	215	220	
Gln	Ser	Gly	Asn	Ala	Ser	Arg	Ser	Arg	Arg	Val						225	230	235	

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 705 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: YES

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Varicella-Zoster-Virus

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(B) STRAIN: Ellen

(viii) POSITION IN GENOME:

(B) MAP POSITION: ORF23

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ATGACACAAC CCGCATCGTC TCGTGTAGTC TTTGATCCCA GCAACCCAC CACATTTTCG	60
GTGGAAGCAA TTGCGGCTTA CACCCCGTT GCTTTAATAC GACTTTTAAA CGCCAGTGGA	120
CCTTTGCAAC CTGGTCACCG TGTGGACATC GCTGATGCCA GAAGCATTTA CACCGTGGGA	180
GCCGCGGCCA GTGCCGCGCG TGCACGCGCT AACCATAATG CAAATACGAT ACGCCGAACG	240
GCCATGTTTG CCGAGACTGA CCCTATGACA TGGTTAAGAC CAACGGTTGG CTTAAAACGT	300
ACGTTTAACC CGCGTATTAT ACGACCACAA CCCCCAAATC CATCCATGAG TTTGGGAATC	360
TCGGGGCCTA CTATATTGCC GCAAAAAACA CAGAGCGCCG ATCAGTCTGC TTTACAACAG	420
CCCGCCGCGT TGGCGTTTTC GGGATCATCC CCGCAACACC CCCACCTCA AACGACGTCG	480
GCATCCGTTG GACAACAGCA ACACGTGGTG TCGGGGTCTT CTGGACAACA ACCGCAACAG	540
GGAGCACAGT CAAGCACTGT CCAGCCAACA ACCGGATCAC CGCCCGCGGC CCAAGGCGTG	600
CCACAGTCTA CCCC GCCCCC AACCCAAAAT ACCCCCCAGG GGGGTAAGGG ACAGACCTTG	660
TCACACACGG GACAATCTGG AAACGCTTCA AGAAGTCGTA GGGTG	705

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Synthetic DNA"

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Varicella-Zoster-Virus
- (B) STRAIN: Ellen

(viii) POSITION IN GENOME:

(B) MAP POSITION: ORF23

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GGAATTCCGG ATGACACAAC CCGCATCGTC TCGTGTA	37
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(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "Synthetic DNA"
- (iii) HYPOTHETICAL: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Varicella-Zoster-Virus
 - (B) STRAIN: Ellen
- (viii) POSITION IN GENOME:
 - (B) MAP POSITION: ORF23

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:
GCTCTAGATT ACACCCTACG ACTTCTTGAA GCGTTTCC

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(2) INFORMATION FOR SEQ ID NO: 5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 34 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "Synthetic DNA"
- (iii) HYPOTHETICAL: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Varicella-Zoster-Virus
 - (B) STRAIN: Ellen
- (viii) POSITION IN GENOME:
 - (B) MAP POSITION: ORF23

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:
GGAATTCCGC GCCTGCAGGT CGACACTAGT GGAT

34

(2) INFORMATION FOR SEQ ID NO: 6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 38 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "Synthetic DNA"
- (iii) HYPOTHETICAL: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Varicella-Zoster-Virus
 - (B) STRAIN: Ellen
- (viii) POSITION IN GENOME:
 - (B) MAP POSITION: ORF23

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GCTCTAGATT ACACCCTACG ACTTCTTGAA GCGTTTCC

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(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Synthetic DNA"

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Varicella-Zoster-Virus
- (B) STRAIN: Ellen

(viii) POSITION IN GENOME:

(B) MAP POSITION: ORF23

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CGGATCCGAT CCCAGCAACC CCACCAC

27

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Synthetic DNA"

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Varizella-Zoster-Virus
- (B) STRAIN: Ellen

(viii) POSITION IN GENOME:

(B) MAP POSITION: ORF23

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GCTCTAGATT ACACCCTACG ACTTCTTGAA GCGTTTCC

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Claims

1. An immunoreactive peptide which shares 90% sequence identity with the AA 12 to 235 region of varicella zoster virus (VZV) VP26 shown in SEQ ID NO:1.
5
2. An immunoreactive peptide which consists essentially of the AA 12 to 235 region of varicella zoster virus (VZV) VP26 shown in SEQ ID NO:1.
10
3. A nucleic acid which encodes the amino acid sequence as claimed in claim 1 or 2.
- 15 4. A nucleic acid which corresponds to the nucleotide sequence depicted in SEQ ID NO:2.
- 20 5. A nucleic acid which hybridizes under stringent conditions with the complement of the nucleotide sequence in SEQ ID NO:2 and encodes a peptide which is recognized by antibodies which are directed against varicella zoster virus (VZV) but not by antibodies which are directed against other herpes-viruses.
- 25 6. An immunoreactive peptide which can be prepared by expressing the nucleic acid as claimed in any one of claims 3 to 5.
- 30 7. An immunochemical method for detecting antibodies against varicella zoster virus (VZV) in a sample using

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an immunoreactive peptide as claimed in one of claims 2 and 6.

8. The use of the nucleic acid as claimed in any one of claims 3 to 5 for detecting varicella zoster virus (VZV) by means of nucleic acid hybridization.

9. A test kit for detecting antibodies against varicella zoster virus (VZV), which comprises an immunoreactive peptide as claimed in any one of claims 1, 2 and 6 and instructions for the use thereof.

10. A test kit for detecting varicella zoster virus (VZV), which comprises the nucleic acid as claimed in any one of claims 3 to 5 and instructions for the use thereof.