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(54) Title: PROCEDURE FOR PREDICTING THE RECURRENCE OF HUMAN CERVICA CARCINOMA			
(57) Abstract A prognostic procedure for predicting the likelihood of recurrence of cervical carcinoma in a patient previously diagnosed as having cervical carcinoma is disclosed. The procedure may be used before the patient begins therapy, such as radiation or surgical therepay, or after the patient completes therapy. In the procedure, a serum or plasma sample taken from the patient is combined with a predetermined amount of substantially pure ICP10 antigen so that any ICP10 antibodies present in the serum or plasma sample bind to the antigen to form antigen-antibody complexes. The amount of these complexes is then determined, to thereby detect the presence of ICP10 antibodies in the serum or plasma sample. The presence of these antibodies serves to indicate that cervical carcinoma is not likely to recur in the patient. In a preferred embodiment of the invention, the amount of antigen-antibody complexes is determined with a non-competitive, heterogeneous immunoassay.			

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PROCEDURE FOR PREDICTING THE RECURRENCE
OF HUMAN CERVICAL CARCINOMA

Technical Field

The present invention relates to a procedure for predicting whether or not cervical carcinoma will recur in a patient who has been
5 previously diagnosed for cervical carcinoma. The procedure may be used either before or after the patient is treated.

Background of the Invention

The recurrence of human cervical carcinoma
10 in a previously diagnosed patient, like the recurrence of many metastatic diseases, is currently an unpredictable event. Combined with this unpredictability problem is the fact that the recurrence of cervical carcinoma is difficult to
15 detect at an early stage, requiring expensive and time-consuming clinical examinations. At present, there is no simple and reliable screening procedure available which aids in predicting the recurrence of cervical carcinoma. The early diagnosis of
20 recurrent disease is, however, an important step in its successful treatment, and the availability of a predictive, or "prognostic", screening procedure which would aid in indicating whether or not cervical carcinoma will recur would greatly
25 facilitate clinical management.

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Research in this area, while of considerable interest, has not heretofore provided a reliable prognostic procedure. Aurelian, L. et al., Science 181, 161 (1973), reported that a crude
5 extract from HEp-2 (human epidermoid carcinoma) cells which had been infected with herpes simplex virus type 2 (HSV-2) for four hours reacted with antibodies in the serum of patients with cervical carcinoma. The antigen in the crude extract to
10 which these antibodies were believed to be directed was designated AG-4, but the procedure described used only a crude cell extract containing the designated AG-4 antigen in combination with a multiplicity of other antigens. Importantly,
15 Aurelian et al. reported that antibodies to this crude AG-4 containing extract were absent in women without recurrent cervical carcinoma, and accordingly suggested that this crude fraction could be used in a prognostic procedure. See also
20 Aurelian, L. et al., Am. J. Epidemiol. 98, 1 (1973).

The unpredictability of this technology was subsequently exemplified by Heise, E. et al., Cancer Res. 39, 4022 (1979), in which a crude AG-4 containing extract was used in a complement fixation
25 assay to screen for AG-4 antibodies in patients which had been previously diagnosed for cervical carcinoma. This study reached an opposite conclusion from that reached by Aurelian et al., tentatively suggesting that the presence of
30 antibodies to the crude fraction might have a favorable prognostic significance. The position of Heise et al. was subsequently supported by Arsenakis, M. et al., The Lancet, 437 (Feb. 21, 1981).

35 There are significant disadvantages in using a crude protein fraction in a prognostic procedure. Because of the presence of contaminating

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proteins, a substantial amount of nonspecific binding occurs. Nonspecific binding renders the prognostic procedure less sensitive because it is more difficult to distinguish the binding event of interest from the background "noise." More seriously, the presence of contaminating proteins increases the chances that the test will result in a false positive. In the prognostic procedure with which the present invention is concerned, false positives produce an erroneous favorable prediction that cervical carcinoma will not recur in the treated patient.

Numerous studies have focused, among other things, on further identification and purification of the AG-4 antigen, which has come to be called the ICP10 antigen. See Aurelian, L. and Strnad, B.C., Cancer Res. 36, 810 (1976); Kawana, T. et al., Gann 69, 589 (1978); Arsenakis, M. and May, J.T., Infection and Immunity 33, 22 (1981); Arsenakis, M. and May, J.T., Br. J. Vener. Dis. 58, 48 (1981); Flanders, R.T. et al., Virus Res. 2, 245 (1985); Swain, M.A. and Galloway, D.A., J. Virology 57, 802 (1986). These studies were conducted for a variety of reasons, but were largely unrelated to the development of a prognostic screening procedure. The identification of a specific antigen of prognostic significance would nevertheless be of considerable value in developing a more sensitive, accurate, and reliable prognostic procedure.

Summary of the Invention

As a result of continued research on prognostic screening procedures, Applicant has discovered that the ICP10 antigen is the specific individual antigen useful in a prognostic procedure for predicting the likelihood of recurrence of cervical carcinoma in a patient previously diagnosed as having cervical carcinoma. By excluding the

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possibility that other, unidentified antigens may have been of prognostic significance in the crude AG4 extracts used in prior prognostic procedures, applicant's development permits the sensitivity of procedures for prognosing the recurrence of cervical carcinoma to be increased, and the likelihood of such procedures producing a false positive to be decreased. In applicant's procedure, a serum or plasma sample taken from such a patient is combined with a predetermined amount of substantially pure ICP10 antigen so that any ICP10 antibodies present in the serum or plasma sample bind to the antigen to form antigen-antibody complexes. The amount of these complexes is then determined, to thereby detect the presence of ICP10 antibodies in the serum or plasma sample. The presence of these antibodies serves to indicate that cervical carcinoma is not likely to recur in the patient.

Stated more particularly, applicant's procedure comprises the following steps:

(a) combining a serum sample taken from a patient previously diagnosed as having cervical carcinoma with a predetermined amount of substantially pure ICP10 antigen, wherein the ICP10 antigen is bound to a solid support, so that ICP10 antibodies present in the serum sample bind to the antigen to form antigen-antibody complexes;

(b) adding labelled antibodies specific for the ICP10 antibodies to form a sandwich comprised of the ICP10 antigen, the ICP10 antibodies, and the labelled antibodies;

(c) separating the solid support from any labelled antibodies not bound to the ICP10 antibodies; and

(d) determining the amount of labelled antibodies bound to the solid support to detect the presence of ICP10 antibodies in the serum sample,

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the presence of these antibodies in the serum sample serving to indicate that cervical carcinoma is not likely to recur in the patient.

5 The following detailed description and examples are provided to more completely explain the present invention. These materials are provided for illustrative purposes only, and are not to be taken as limiting applicant's invention.

Detailed Description of the Invention

10 In one embodiment of this invention, the serum or plasma sample is taken from a patient prior to the completion of the patient's prescribed treatment for cervical carcinoma, with the procedure serving as an indicator of the effectiveness of the
15 treatment in preventing recurrent disease. In another embodiment, the serum or plasma sample is taken from the patient after the completion of the patient's treatment for cervical carcinoma. In this latter embodiment, the procedure serves to indicate
20 the degree of post-treatment monitoring in the form of clinical examinations which the patient should receive.

The manner by which the presence of ICP10 antibodies in the plasma or serum sample is detected
25 is not critical for the purposes of the present invention. A number of suitable immunoassays are available which can be routinely adapted for use with the present invention by those skilled in the art. See, e.g., Immunology: Basic Processes, 160-
30 75 (J.A. Bellanti, Ed. 1985). Some of these immunoassays may be classified as being either competitive or non-competitive. In an exemplary competitive assay, the unknown antibody to be measured competes for binding on an antigen with a
35 known quantity of labelled antibody. Greater binding of the labelled antibody indicates that a lesser quantity of the unknown antibody is present.

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In an exemplary non-competitive assay, the unknown antibody to be measured binds to an antigen, and a labelled antibody (e.g., an antibody carrying a label) binds to the unknown antibody. Greater
5 binding of the labelled antibody indicates that a greater quantity of the unknown antibody is present.

Some of the suitable assays may also be classified as being either heterogeneous or homogeneous. A heterogeneous assay requires that
10 bound label (label bound to the antibody or antigen to be measured) and unbound label be detached in a separation step. In a heterogeneous assay, either the antigen or antibody is normally bound to a solid support to facilitate this separation. Conventional
15 solid supports include latex particles, container surfaces such as the wall of a microtiter plate well, and glass beads. Conventional means for binding the antigen or antibody to the solid support include both the adsorption and the covalent linkage
20 of the antigen or antibody to the support. Homogeneous assays do not involve a separation step, but instead rely on the fact that, in such assays, the signal produced by the label when the label is involved in an antibody-antigen complex differs from
25 the signal produced by the label when the label is not involved in an antibody-antigen complex.

Suitable labels for use in such immunoassays include radiolabels such as Sulfur 35, Iodine 125, and Iodine 131, enzyme labels such as
30 horseradish peroxidase and alkaline phosphatase, and fluorescent labels such as fluorescein. Suitable assays for practicing the invention thus include radioimmunoassays, enzyme immunoassays, and fluoroimmunoassays. For radiolabels, iodine 131 and
35 iodine 125 are preferred for their high activity,

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short half-life, and ease of binding to a preformed antigen. Assays used for the present invention are preferably noncompetitive, heterogeneous immunoassays.

5 The phrase "substantially pure ICP10 antigen" means ICP10 antigen provided in a purity greater than the level of purity obtained with the technique disclosed in Aurelian, L. et al. Science 181, 161 (1973) and Aurelian, L. et al., Am. J. Epidemiol 98, 1 (1973). More preferably, the
10 substantially pure ICP10 antigen used in practicing the present invention is ICP10 antigen substantially free of nonsoluble proteins and particulate matter, such as cell membranes and their associated
15 proteins. Unexpectedly, such a preparation provides a prognostic procedure which is more sensitive and produces fewer false positives than prior
20 procedures. This result is unexpected because it was not heretofore known that the ICP10 antigen was the specific individual antigen of prognostic
25 significance in such procedures. The ICP10 antigen preferably comprises a herpes simplex virus type two (2) induced ICP10 antigen. Particularly preferred is herpes simplex virus type 2 ANG/63 strain, which
30 was deposited on November 10, 1987 under the Budapest Treaty at the American Type Culture Collection, and has been assigned number VR2191. While the terms "AG4" and "ICP10" are now often used interchangeably, for clarity applicant herein uses
35 the term "ICP10" only in association with the substantially pure antigen, because the term "AG4" was originally used in association with a crude, less pure, fraction.

 In addition to the technical and
35 scientific literature which may be referred to facilitate the practice of the present invention, with which those skilled in the art will be well

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familiar, there are also numerous patent references available which concern immunometric assays. A few examples of these references are U.S. Patent No. 4,659,678 to Forrest et al., titled "Immunoassay of Antigens," U.S. Patent No. 4,376,110 to David et al., titled "Immunometric Assays Using Monoclonal Antibodies," U.S. Patent No. 4,275,149 to Litman et al., titled "Macromolecular Environment Control in Specific Receptor Assays," U.S. Patent No. 4,233,401 to Yoshida et al., titled "Antienzyme Homogeneous Competitive Binding Assay," U.S. Patent No. 4,233,402 to Maggio et al., titled "Reagents and Method Employing Channeling," and U.S. Patent No. 4,230,797 to Boguslaski et al., titled "Heterogeneous Specific Binding Assay Employing a Coenzyme as Label." The disclosures of these references are to be incorporated herein by reference.

The following examples are provided to further illustrate the present invention. While they are given in considerable detail, numerous variations on these examples can be made by those skilled in the art. Accordingly, these examples are to be considered illustrative of the present invention rather than restrictive thereof.

Example 1

Preparation of Substantially Pure ICP10 Antigen

HEp-2 cell monolayers (about 4×10^7 cells) in 150 square centimeter flask cultures are infected with herpes simplex virus type 2 (HSV-2), ANG/63 strain, at a multiplicity of infection = 5.0. After one hour virus attachment at 37°C, the unattached virus suspension is aspirated and the cell monolayers are overlaid with 15 milliliters Dulbecco's modified minimal essential medium supplemented with 2% (volume/volume) fetal calf

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serum, penicillin (100 units/milliliter) and streptomycin (100 micrograms/milliliter). The cultures are incubated at 37°C for 8 hours. To harvest the cells, the cultures are washed twice
5 with 0.01 Molar phosphate buffered 0.85% NaCl, pH 7.2 (PBS-A) and the washed cells are scraped into 10 milliliters of PBS-A. The cells are pelleted (450 x gravity, 10 minutes) and resuspended in 2 volumes of hypotonic buffer (50 millimolar HEPES, pH 7.5, 1
10 millimolar MgCl₂ and 2 millimolar DTT) at 4°C. The resuspended cells are homogenized with 25-50 strokes in a Dounce homogenizer and the lysed cells are centrifuged (11,000 x gravity, 30 minutes, 4°C). The supernatant containing ICP10 is dialyzed against
15 three separate 500 milliliter changes of hypotonic buffer over a 3-5 hour period at 4°C. To the dialyzed supernatant 40% (weight/volume) streptomycin sulfate solution is added dropwise until the final concentration is 1% (weight/volume)
20 streptomycin sulfate. The mixture is stirred for 15 minutes at 4°C and then centrifuged at 11,000 x gravity for 15 minutes at 4°C. Solid ammonium sulfate is added to the supernatant to make the supernatant 45% (weight/volume) ammonium sulfate.
25 The mixture is stirred for 30 minutes at 4°C and then centrifuged at 11,000 x gravity for 15 minutes at 4°C. The supernatant is dialyzed against 3 changes (500 milliliters each) of hypotonic buffer over a time period of 3 hours at 4°C and stored at -
30 70°C. The ICP10 antigen thus produced is a polypeptide with a molecular weight of 143 to 165 Kilodaltons, as measured by polyacrylamide gel electrophoresis.

For control, mock-infected HEp-2 cell
35 monolayers are treated in the identical manner to obtain a partially purified control extract (AgH) of the cells.

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Example 2Alternate Procedure for the Preparation
of Substantially Pure ICP10 Antigen

This example illustrates that

5 substantially pure ICP10 antigen suitable for use in
practicing the present invention may be obtained by
different means. In this example, the antigen is
isolated from 75 square centimeter flask cultures of
HEp-2 cells infected with HSV-2 (ANG/63 strain)
10 virus at a multiplicity of infection = 0.5 plaque
forming units per cell. After 1.5 hours virus
attachment at 37°C, the infected cultures are washed
once to remove unattached virus and then overlaid
with 5 milliliters Dulbecco's modified minimal
15 essential medium containing 2% fetal calf serum.
After an additional 4 hours of incubation at 37°C,
the cells from each flask culture are washed twice
with 0.01 Molar phosphate buffered saline (PBS),
scraped into 5 milliliters of PBS, pelleted by
20 centrifugation (450 x gravity for 5 minutes) and
washed two additional times with 10 milliliters PBS
per 1×10^7 cells. The final cell pellet is
resuspended in PBS (1×10^7 cells/milliliters) and
the cells lysed by freezing and thawing three times
25 and by sonication for 1 minute using a Branson
sonifier at full power. The cell lysate containing
ICP10 is cleared by centrifugation at 100,000 x
gravity for 1 hour. The cleared supernatant is used
as a source of purified ICP10.

30 The procedure described in example 1 is
preferred.

Example 3ELISA Procedure Using Substantially
Pure ICP10

35 This example illustrates a particular
heterogeneous, non-competitive enzyme immunoassay
called an enzyme-linked immunosorbent assay (ELISA)
useful for practicing the present invention.

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50 microliters of ICP10 antigen prepared according to the procedure set forth in example 1 above containing 6.25-25 micrograms protein is added to each well in a 96 well plate. The plates are

5 incubated for 1 hour at 20°C or 18 hours at 4°C to allow attachment of the antigens to the bottom of the well. The wells are washed 4 times with 100 microliters 0.01 Molar phosphate buffered saline (8 grams NaCl, 0.2 grams, KH_2PO_4 , 2.9 grams Na_2HPO_4 , 0.2

10 grams KCL, H_2O to 1000 milliliters) supplemented with 0.05% (volume/volume) Tween 20 (PBS-Tween 20), and treated with 200 microliters PBS supplemented with 5% (volume/volume) bovine serum albumin (PBS-BSA) for 2 hours at 25°C, or 18 hours at 4°C. Each well

15 is washed once with 100 microliters PBS-Tween 20 and then treated with 50 microliters of patient's serum diluted in PBS-BSA. After 2 hours incubation at 25°C each well is washed 5 times with 100 microliters PBS-Tween 20 to remove unadsorbed serum

20 antibodies. To each well is added 100 microliters of 0.1% (volume/volume) phenylhydrazine in PBS. The 96 well plate is incubated for 1 hour at 25°C to block endogenous peroxidase. After washing each well 4 times with 100 microliters PBS-Tween 20, 100

25 microliters of goat anti-human IgG, IgM, IgA antibodies conjugated with horseradish peroxidase are added to each well. After incubation for 2 hours at 25°C, each well is washed 5 times with 100 microliters PBS-Tween 20. A fresh ortho-diansidine

30 substrate solution is prepared in the following manner: To 1 milliliter of 50% methanol in H_2O is added 10 milligrams of ortho-diansidine, and 250 microliters of the resulting solution is added to 10.76 milliliters of Tham citrate buffer (0.3 Molar

35 Tris adjusted to pH 6.0 with 1 Molar citric acid) supplemented with 4.5 microliters of fresh hydrogen peroxide. 100 microliters of the above ortho-

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diansidine substrate solution are added to each well and the 96 well plate is incubated for 30 minutes at 25°C. The ortho-diansidine and horseradish peroxidase reaction is stopped by adding 20
5 microliters of 2% (weight/volume) sodium azide per well. The absorbance of the color reaction in each well is measured spectrophotometrically at 450 nanometers. The absorbance data for each test serum reacted with ICP10 are compared to absorbance data
10 for each test serum reacted with control HEp-2 cell antigens (uninfected cell antigens).

Example 4

ICP10 ELISA As A Prognostic Procedure

The ICP10 ELISA procedure described in
15 example 3, and a standard Western blot procedure described in Flanders, Kucera, Raben, Ricardo, Virus Research 2:245 (1985), were conducted using human cervical carcinoma sera, a positive reference serum (rabbit ICP10 antibodies) and a negative reference
20 serum (rabbit preimmune). The results (Table 1) showed good correlation between reactivity of human cervical carcinoma sera with ICP10 in both ELISA and Western blot assays.

TABLE 1

Detection of Ag4/ICP10 Antibodies in Sera From Cervical
Carcinoma Patients Measured by ELISA and Western Blot Assays

Assays	Reactivity of Human Cervical Carcinoma Sera With:						Reference Sera		
	PATIENT A		PATIENT B		PATIENT C		Pos		Neg
	ICP10	AgH	ICP10	AgH	ICP10	AgH	ICP10	AgH	ICP10 AgH
ELISA	+	-	-	-	+	-	+	-	-
Western blot	+	-	-	-	+	-	+	-	-

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The human cervical carcinoma sera did not react with the control partially purified extract (AgH). These data confirm that some, but not all, human cervical carcinoma patients possess serum antibodies reactive with HSV-2 strain ANG/63 induced ICP10. In view of results previously obtained in prognostic studies using the crude AG4 extract, these data indicate that the presence of ICP10 serum antibodies correlates with an excellent prognosis in treated patients, and that the absence of detectable ICP10 antibodies correlates with a poor prognosis in treated patients. The correlation between the ELISA assays and the Western Blot assays shown in Table 1 confirms the prognostic significance of the ICP10 antigen, specifically and individually, in the substantially pure ICP10 antigen preparation used. Thus, the ICP10 antigen purified to homogeneity is useful in procedures for predicting the recurrence of cervical carcinoma, as described above, as the Western blot data confirm that any other proteins present in applicant's substantially pure ICP10 preparation are not of prognostic significance.

The foregoing examples are illustrative of the present invention rather than restrictive thereof, with the scope of the invention being defined by the following claims. Equivalents of the claims are to be included therein.

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THAT WHICH IS CLAIMED IS:

1. A prognostic procedure for predicting the likelihood of recurrence of cervical carcinoma in a patient previously diagnosed as having cervical carcinoma, comprising the steps of

5 (a) combining a serum or plasma sample taken from said patient with a predetermined amount of substantially pure ICP10 antigen so that any ICP10 antibodies present in said serum or plasma sample bind to said antigen to form antigen-antibody complexes; and then

10 (b) determining the amount of said complexes to detect the presence of ICP10 antibodies in said serum or plasma sample, the presence of said antibodies serving to indicate that cervical carcinoma is not likely to recur in said patient.

2. A procedure according to Claim 1, wherein the amount of said complexes is determined with a competitive assay.

3. A procedure according to Claim 1, wherein the amount of said complexes is determined with a non-competitive assay.

4. A procedure according to Claim 1, wherein the amount of said complexes is determined with a homogeneous assay.

5. A procedure according to Claim 1, wherein the amount of said complexes is determined with a heterogeneous assay.

6. A procedure according to Claim 1, wherein the amount of said complexes is determined with a radioimmunoassay.

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7. A procedure according to Claim 1, wherein the amount of said complexes is determined with an enzyme immunoassay.

8. A procedure according to Claim 1, wherein the amount of said complexes is determined with a fluoroimmunoassay.

9. A procedure according to Claim 1, wherein said serum or plasma sample is taken from said patient prior to the completion of said patient's treatment for cervical carcinoma.

10. A procedure according to Claim 1, wherein said serum or plasma sample is taken from said patient after the completion of said patient's treatment for cervical carcinoma.

11. A procedure according to Claim 1, wherein said substantially pure ICP10 antigen comprises a herpes simplex virus type 2 ANG/63 strain induced ICP10 antigen.

12. A prognostic procedure for predicting the likelihood of recurrence of cervical carcinoma in a patient previously diagnosed as having cervical carcinoma, comprising the steps of

5 (a) combining a serum or plasma sample taken from said patient with a predetermined amount of substantially pure ICP10 antigen so that any ICP10 antibodies present in said serum or plasma sample bind to said antigen to form antigen-antibody
10 complexes; and then

(b) determining the amount of said complexes with a heterogeneous, non-competitive assay to detect the presence of ICP10 antibodies in

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15 said serum or plasma sample, the presence of said
antibodies serving to indicate that cervical
carcinoma is not likely to recur in said patient.

13. A procedure according to Claim 12,
wherein said substantially pure ICP10 antigen
comprises a herpes simplex virus type 2 ANG/63
strain induced ICP10 antigen.

14. A prognostic procedure for predicting
the likelihood of recurrence of cervical carcinoma
in a patient previously diagnosed for cervical
carcinoma, comprising the steps of:

5 (a) combining a serum sample taken from
said patient with a predetermined amount of
substantially pure ICP10 antigen wherein said ICP10
antigen is bound to a solid support so that ICP10
antibodies present in said serum sample bind to said
10 antigen to form antigen-antibody complexes;

(b) adding labelled antibodies specific
for said ICP10 antibodies to form a sandwich;

(c) separating said solid support from
any labelled antibodies not bound to said ICP10
15 antibodies; and

(d) determining the amount of labelled
antibodies bound to said solid support to detect the
presence of ICP10 antibodies in said serum sample,
the presence of said antibodies in said serum sample
20 serving to indicate that cervical carcinoma is not
likely to recur in said patient.

15. A procedure according to Claim 14,
wherein said labelled antibody carries a label
selected from the class consisting of radiolabels,
enzyme labels, and fluorescent labels.

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16. A procedure according to Claim 14, wherein said solid support is selected from the class consisting of particles, container surfaces, and plates.

17. A procedure according to Claim 14, wherein said ICP10 antigen is either adsorbed or covalently linked to said solid support.

18. A procedure according to Claim 14, wherein said serum or plasma sample is taken from said patient prior to the completion of said patient's treatment for cervical carcinoma.

19. A procedure according to Claim 14, wherein said serum or plasma sample is taken from said patient after the completion of said patient's treatment for cervical carcinoma.

20. A procedure according to Claim 14, wherein said substantially pure ICP10 antigen comprises a herpes simplex virus type 2 ANG/63 strain induced ICP10 antigen.

INTERNATIONAL SEARCH REPORT

International Application No. **PCT/US89/00578**

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC(4): GO1N 33/53, 33/543, 33/58, 33/566		
U.S. CL.: 435/7; 436/501, 518		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
U.S.	435/7; 436/501, 518; 530/350, 811, 828	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
Computer Search: APS, CAS, BIOSIS		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category [*]	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
Y	Chemical Abstracts, volume 104, No. 1 issued January 6, 1986, Columbus, Ohio Iwasaka et al., "The cervical tumor- associated antigen (ICP-10/AG-4) is encoded by the transforming region of the genome of herpes simplex virus type 2.", see page 140, abstract 1513x.	1-20
Y	Chemical Abstracts, volume 103, No. 9 issued September 2, 1985, Columbus, Ohio, Flanders et al., "Immunologic characterization of herpes simplex virus type 2 antigens ICPl0 and ICSP 11/12.", see page 494, abstract 69473u.	1-20
[*] Special categories of cited documents: ¹⁰ "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
11 MAY 1989	19 MAY 1989	
International Searching Authority	Signature of Authorized Officer	
ISA/US	<i>Janelle Graeter</i> JANELLE GRAETER	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Proceedings of the National Academy of Sciences, volume 82 issued December, 1985, Washington, D.C., Hayashi et al., "Multistep transformation by defined fragments of herpes simplex virus type 2 DNA: Oncogenic region and its gene product", pages 8493-8497, see pages 8496-8497.	1-20
A	Journal of Virology, volume 42(1) issued April, 1982, Washington, D.C., Lewis et al., "Detection of Herpes Simplex Virus Type 2 Glycoproteins Expressed in Virus-Transformed Rat Cells", pages 275-281, see Abstract, pages 279-281.	1-20
Y	Basic and Clinical Immunology, 1980, Lange Medical Publications (Los Altos, California), Fudenberg et al., ed., "Clinical Laboratory Methods for Detection of Antigens and Antibodies", see pages 365-367, 370-373.	1-20