(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 September 2014 (18.09.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/138795 A1

(51) **International Patent Classification**:
C12Q 1/26 (2006.01) *C12Q 1/68* (2006.01)

(21) **International Application Number**:
PCT/AU2014/000254

(22) **International Filing Date**:
13 March 2014 (13.03.2014)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
2013900950 13 March 2013 (13.03.2013) AU

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2014/138795 A1

(54) **Title**: DETECTION OF INFECTIOUS PATHOGEN AND CONTAGIOUS DISEASE

(57) **Abstract**: The invention relates to methods and devices for determining whether an individual has an infectious pathogen or contagious disease, including, but not limited to HSV-2 and genital herpes.

Detection of infectious pathogen and contagious disease

Field of the invention

The invention relates to the detection of infectious pathogen and contagious disease, especially sexually transmitted disease and related pathogens, and to devices and kits for enabling detection of same.

Background of the invention

Reference to any prior art in the specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in Australia or any other jurisdiction or that this prior art could reasonably be expected to be ascertained, understood and regarded as relevant by a person skilled in the art.

The increasing prevalence of sexually transmitted diseases (STD) is of significant global concern. One reason for increased prevalence is that an infected individual may not be aware that he/she has transmissible disease at the time of intercourse. This is because many STDs have intermittent periods of remission, dormancy or latency, followed by periods of activation where even an asymptomatic individual may contain transmissible actiological agent. This increases the likelihood of spread of disease from individual to individual.

The example of the STD, genital herpes neatly illustrates the relevant principle referred to above. This disease arises from infection with Herpes simplex virus -1 (HSV-1) or Herpes simplex virus -2 (HSV-2), and it is characterised by periods of activation and latency. In a latent state, the virus resides in neuronal ganglia, and the infected individual does not display symptoms of infection. When activated, virus particles may generally be detected in epithelia, and an infected individual may present with epithelial ulceration, headaches, malaise and muscular aches, in addition to localised itching, swollen lymph nodes, neuralgic pain and difficulty urinating. Importantly some individuals are asymptomatic at this stage, or otherwise do not have clear manifestations of disease, and yet shed contagious viral particles that may be transmitted by skin to skin or mucous membrane contact. It is believed that these individuals account for approximately 70% of HSV-2 transmission.

Clearly the key to minimising transmission of STDs is to enable an infected individual to identify whether he/she has contagious disease so that the individual can make an informed decision on this basis as to whether to engage in intercourse. Particular individuals of concern are those having a lifelong infection that cycles through periods of activation and latency and who tend to have few or no symptoms of disease when their infection is in a period of activation (i.e. when the agent causing the pathology has become transmissible or contagious). One example is a HSV-2-infected individual who has active transmissible disease, but few or no symptoms or manifestations of HSV-2 infection.

It would be useful to provide the aforementioned individuals with means or methods to enable them to determine, prior to intercourse, whether the individual is likely to transmit disease to a partner. One example of such an individual is a person with a HSV-2 infection who has active, transmissible disease, and who may be asymptomatic or otherwise display few manifestations of active disease.

Many of the aetiological agents of STDs are associated with significant morbidity or mortality, or otherwise increase the likelihood of opportunistic infection with other agents that are associated with significant morbidity or mortality. For example, serious complications of HSV-2 infection include meningitis and encephalitis, as well as the development of neonatal herpes in babies if the virus is transmitted to the infant during vaginal delivery. HSV-2 infection also increases the risk of HIV acquisition by 3- to 4-fold.

Most STDs, or the relevant symptoms, may be treated by various drugs. For example, genital herpes can be managed by acyclovir, valaciclovir and famciclovir, all of which suppress viral replication. However, the efficacy of treatment, at least in terms of minimisation of symptoms, is largely dependent on the stage of the infection at which the therapy is given. Early treatment minimises the likelihood of occurrence of some symptoms of disease. Later treatment may result in the emergence of more symptoms or potentially serious complications of disease, or the duration of either.

Of particular concern are those STDs that are not manifestly apparent in an individual until well after the relevant aetiological agent has had opportunity to establish widespread infection. It would be particularly useful to determine whether an individual is likely to develop symptoms of disease long before the symptoms become apparent, as this would allow early

therapeutic intervention, potentially minimising the duration or extent of a symptomatic phase of the STD.

Summary of the invention

The invention seeks to minimise one or more of the aforementioned problems and/or to provide means and/or methods for addressing an aforementioned need and in one embodiment provides a method for determining whether an individual comprises an infectious pathogen including:

- determining whether the individual contains an increased expression or activity of an enzyme of the individual that has substrate specificity for an amino acid;

wherein the individual is determined to have an infectious pathogen where the individual is determined to contain an increased expression or activity of the enzyme.

In another embodiment there is provided a method for determining whether an individual has a contagious or transmissible disease including:

- determining whether the individual contains an increased expression or activity of an enzyme of the individual that has substrate specificity for an amino acid;

wherein the individual is determined to have a contagious or transmissible disease where the individual is determined to contain an increased expression or activity of the enzyme.

In another embodiment there is provided a method for determining whether an individual has an infectious pathogen in the form of infectious HSV-2 including:

- providing a test sample of vaginal mucus in the form of a sample obtained from an individual in whom the presence of infectious HSV-2 is to be determined;

- determining whether the test sample contains an increased level of kynurenine relative to the level of kynurenine in a control sample in the form of a vaginal sample of an individual known not to have HSV-2 infection and/or another infection;

wherein the individual is determined to have infectious HSV-2 where the test sample is determined to contain an increased amount of kynurenine.

In another embodiment there is provided a method for determining whether an individual has contagious genital herpes including:

- providing a test sample of vaginal mucus in the form of a sample obtained from an individual in whom the presence of contagious genital herpes is to be determined;

- determining whether the sample contains an increased level of kynurenine relative to the level of kynurenine in a control sample in the form of a vaginal sample of an individual known not to have HSV-2 infection and/or another infection;

wherein the individual is determined to have contagious genital herpes where the test sample is determined to contain an increased amount of kynurenine.

In another embodiment there is provided a method for preventing the appearance of symptoms of disease in an individual, including the step of:

- providing an individual who has been determined to have contagious disease or an infectious pathogen according to a method described above;

- administering a therapeutic compound to the individual to minimise the appearance of symptoms of the disease in the individual;

thereby minimising the appearance of symptoms of disease in the individual.

In another embodiment there is provided a method for minimising the risk of transmission of a contagious disease from an individual to another individual including the step of:

- providing an individual who has been determined to have contagious disease or an infectious pathogen according to a method described above;

- applying means to the individual to prevent transmission of the infectious pathogen to another individual;

thereby minimising the risk of transmission of contagious disease from the individual.

In another embodiment there is provided a device for use in determining whether an individual contains a contagious disease or infectious pathogen, the device including means for determining whether the individual contains an increased expression or activity of an enzyme of the individual that has substrate specificity for an amino acid.

In another embodiment there is provided a use of a means for determining whether an individual contains an increased expression or activity of an enzyme of the individual that has substrate specificity for an amino acid in the manufacture of means for determining whether an individual contains a contagious disease or infectious pathogen.

In another embodiment there is provided a device including:

- a solid phase

- a reagent attached to the solid phase for detecting the expression or activity of an enzyme of an individual that has substrate specificity for an amino acid.

In another embodiment there is provided a kit including:

- a reagent attached to the solid phase for detecting the expression or activity of an enzyme of an individual that has substrate specificity for an amino acid;

- written instruction for use of the kit in the method described above.

In another embodiment there is provided a device comprising means for use in executing a method according to any one of the above described embodiments.

Brief description of the drawings

Figure 1.

(A) Relative HSV-2 mRNA expression in the vagina of mice infected with 10^5 plaque-forming units (PFU) HSV-2 for 0, 1, 3, 5 and 7 days. HSV-2 mRNA was expressed relative to levels apparent in the vagina at d1 post-infection (p.i.). Data shown are the mean ± SEM of at least 5 mice per group and is representative of 2 independent experiments.

(B) Vaginal pathology of mice infected with 10^5 (PFU) HSV-2 over a 10 day time course, as quantified by the disease score as follows: 0, no signs of infection; 1, slight redness of genital area; 2, redness and swelling of external vagina; 3, severe swelling of the external vagina and hair loss in the surrounding area; 4, ulceration, severe swelling and redness of the vaginal tissue; and 5, continued ulceration, swelling and redness, occasionally accompanied by paralysis of the hind legs (mice were euthanised at this point). Data are expressed as the mean ± SEM of 4 independent experiments of 5 to 12 mice each.

(C) Representative immunofluorescent staining for IDO (red, Alexa Fluor 594) and nuclei (blue, DAPI) in a vaginal cross-section from a mouse infected as in (A) with 10^5 PFU of HSV-2 for 7 days. No positive IDO staining was observed in mock-infected mice or sections stained with the rabbit IgG isotype Ab (not shown).

(D) Kyn concentration in the vaginal mucus of mice infected with 10^5 PFU of HSV-2 for 0, 1, 3, 5, 7 and 10 days. Data are expressed as the mean ± SEM of 4 mice per group and is representative of 2 separate experiments.

Detailed description of the embodiments

Further aspects of the present invention and further embodiments of the aspects described in the preceding paragraphs will become apparent from the following description, given by way of example and with reference to the accompanying drawings.

It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

As used herein, except where the context requires otherwise, the term "comprise" and variations of the term, such as "comprising", "comprises" and "comprised", are not intended to exclude further additives, components, integers or steps.

As referred to above, many diseases, especially sexually transmitted diseases (STD) have intermittent periods of remission, dormancy or latency, followed by periods of active disease. Individuals with active disease may present with some of, all of, or none of the symptoms of the disease.

Of particular concern in this invention are individuals who contain an infectious pathogen and who do not clearly present with symptoms of active disease. '*Infectious pathogen*' generally refers to a disease-causing agent that exists in a form in an infected individual that enables the agent to establish disease in a non-infected individual (i.e. a recipient) when transmitted from an infected individual to the recipient, for example by skin, mucous membrane, or body fluid contact, for example, as may occur during intercourse. '*Non-infectious pathogen*' generally refers to an agent that exists in a form in an infected individual that does not enable the agent to be transmitted from infected individual to a recipient, or if transmitted, does not enable the agent to establish a viable infection or disease in the recipient.

A single type of disease-causing agent may exist as an infectious pathogen, or as a non-infectious pathogen in an infected individual. A pathogen is generally an infectious pathogen when the infected individual has active disease. Where the individual has non-active disease, the pathogen is generally non-infectious.

As an example of the above, some individuals infected with Herpes simplex virus type 2 (HSV-2) may have periods of active disease, followed by periods of remission. Where there is active disease, the HSV-2 particles represent an infectious pathogen because they are transmissible as between infected individual and recipient and they are capable of establishing infection and one or more symptoms of disease in the recipient. Where there is non-active disease (i.e. remission), the HSV-2 particles represent a non-infectious pathogen because the particles are not transmitted between infected individual and non-infected individual, or otherwise the particles do not establish disease in the latter.

There may be one or more factors that define whether a disease-causing agent exists in the form of an infectious pathogen or non-infectious pathogen. One factor may be tissue location. For example, some pathogens, such as HSV-2 are present in the epithelium during active disease only. In non-active disease, HSV-2 is found in neuronal bodies only. Location in the epithelium is relevant as to whether the pathogen is infectious because the epithelium is the

primary point of contact between individuals when there is skin to skin or mucous membrane to membrane contact.

Another factor may be whether the disease-causing agent has expression of the necessary complement of genes for enabling the agent to exist in viable form extracellularly, and/or to infect the cells of a recipient tissue. In this context, some disease-causing agents that are non-infectious pathogens may not contain an essential protein for infecting a target cell through a specific cell surface receptor, or might not otherwise contain the necessary proteins for preventing extracellular degradation.

The invention described herein is concerned with determining whether an individual contains an infectious pathogen. Importantly, the invention enables an infected individual to determine whether, at the time of practice of the invention, the individual has a disease-causing agent that will be transferred to, and establish infection in an individual should there be contact between the individual and the recipient shortly after practice of the invention. Further the invention also enables an infected individual to determine whether contact with another individual (i.e. a recipient) prior to practice of the invention is likely to have transmitted a disease-causing agent, or established disease in the recipient. In this context it will be understood that the invention is distinguished from merely detecting whether an individual contains a pathogen, infectious or otherwise. In particular, it will be understood that the invention is distinguished from detecting whether an individual contains a pathogen that at some later time could become an infectious pathogen. Consistent with the invention, generally the invention will find application among individuals who, prior to practice of the invention, are known to be infected with a disease-causing agent, and the objective among these individuals is to determine whether they are likely to transmit disease to another individual.

As described herein, the inventors have found that enzymes that degrade amino acids are useful as a biomarker for the presence of infectious pathogens. While not wanting to be bound by hypothesis, the inventors have recognised that either or both of the expression and activity of these enzymes is induced in an infected individual when a disease-causing agent transitions from a non-infectious pathogen to an infectious pathogen. It is believed that the induction of these enzymes constitutes one element of the host response to activation of a pathogen. In one example the aforementioned transition requires protein synthesis and it is considered that by increasing

the activity or expression of these enzymes, the host is able to deprive the pathogen of supply of essential amino acids for transition from a non-infectious to infectious state.

The above finding is particularly important where the individual is asymptomatic for active disease and yet contains an infectious pathogen, because it ostensibly establishes the level of expression or activity of the relevant enzyme as a biomarker of the infectious state of the disease-causing agent in the individual. Put in other words, with practice of the invention it becomes possible to determine whether an individual – who may not present with any symptoms of the relevant disease – is nonetheless likely to transmit or otherwise establish disease in another.

It is believed that with practice of the invention it will become possible to minimise the spread of transmissible disease and the underlying infectious agent, thereby decreasing the incidence of disease in a given population. In this context it is believed that the invention will be particularly advantageous for minimising the prevalence of STDs.

Another advantage from the practice of the invention is to minimise the symptoms and potentially serious complications of disease by facilitating earlier therapeutic intervention. In more detail, in many diseases there is a connexion between the disease state (in terms of development of symptoms) and infective potential of the disease-causing agent. Specifically, individuals who have infectious pathogens may have asymptomatic disease that, without therapeutic intervention, could develop into the full spectrum of symptoms of the disease. In this context, the identification of an asymptomatic individual as having infectious pathogen also identifies the individual as a candidate for therapeutic intervention, to prevent the development of symptoms or complications of disease in the individual, long before these hallmarks of disease are manifested.

In one embodiment there is provided a method for determining whether an individual has an infectious pathogen, or whether an individual has contagious or transmissible disease. As generally known in the art, contagious disease is a disease that is capable of spreading from individual to individual by infection with a disease-causing agent. According to the invention, an individual having contagious or transmissible disease is generally an individual who has an infectious pathogen. Individuals who have non-infectious pathogen (generally individuals who do not have active disease) generally do not have contagious disease. These individuals may

develop contagious disease should they develop infectious pathogen. As an example, an individual in remission for genital herpes develops contagious, transmissible disease when the individual develops infectious pathogen in the form of HSV-2 particles that are located in epithelial cells.

The method includes the following steps:

providing an individual in whom the presence of infectious pathogen is to be determined;

determining whether the individual contains an increased expression or activity of an enzyme of the individual that has substrate specificity for an amino acid;

wherein the individual is determined to have an infectious pathogen where the individual is determined to contain an increased expression or activity of the enzyme.

As discussed, an important advantage of the invention is that the methods described herein may be applied to an individual irrespective of whether the individual contains symptoms of the disease controlled by the relevant pathogen. Therefore, in certain embodiments, the individual is asymptomatic for the disease i.e. does not have a symptom that is normally associated with an individual that is infected with the pathogen. In other embodiments, the individual may have some or all of the symptoms or complications of the disease.

In one embodiment, the method is applied for the purpose of determining whether the individual has contagious genital herpes, or for determining whether an individual has infectious HSV -2. In these embodiments, the individual may have none of, or some of, or all of the following symptoms: epithelial ulceration, headaches, fever, malaise and muscular aches, localised itching, swollen lymph nodes, neuralgic pain and difficulty urinating.

In certain embodiments it may not be possible to directly detect the presence of pathogen –whether infectious or otherwise - in the relevant tissue. '*Direct detection*' refers to detection of the pathogen itself. Examples of direct detection include detection of the pathogen or component thereof by an agent that binds to one or more components of the pathogen, for example by an agent that is an antibody, polynucleotide or some other chemical that binds to the pathogen. In contrast, '*indirect detection*' refers to the detection of a chemical species, generally a host factor, that does not form a component of the pathogen itself. Thus, in certain embodiments, the

pathogen is contained in the individual in an amount that is insufficient to enable direct detection of the pathogen in the individual. In particular embodiments the pathogen may be detected in tissue regions where the pathogen is generally considered to be non-infectious, but not directly detected in tissue regions where, if present, the pathogen would be considered to be infectious. As an example relevant to genital herpes, in certain embodiments, infectious pathogen is detected according to the method of the invention in an infected individual in circumstances where the pathogen cannot be directly detected in epithelium. In these embodiments it is believed that induction of the relevant amino acid degrading enzyme occurs prior to synthesis of infectious pathogen in the epithelial tissue.

Typically the enzyme that is to be measured or detected according to the invention is one that has substrate specificity for an amino acid that is an essential amino acid. An essential amino acid is one which the body does not produce or has limited capacity to produce. These amino acids are provided in the body through diet. These enzymes are considered to be particularly important, because as discussed above, it is believed that these form part of the host response to re-activation of disease as a pathogen transitions from a non-infectious to an infectious phenotype. More particularly, it is believed that these enzymes deplete the host pool of otherwise available amino acid residues that are essential for formation of infectious pathogen. By depleting amino acids that are essential amino acids, the host response tends to minimise the number of infectious particles produced during disease reactivation. Examples of enzymes are those having substrate specificity for tryptophan and arginine.

The invention recognises that the connexion between expression and activity of the amino acid-metabolising enzymes and the formation of infectious pathogen means that the former can be used as a biomarker of the latter – i.e. the induction or increase in expression or activity of the enzyme is a marker of the formation or presence of infectious pathogen. Importantly, the invention recognises that the change in expression profile of these amino acid – scavenging enzymes, as disease reactivation occurs during transition from non-infectious to infectious phenotype, long precedes the development of symptoms that generally present in contagious disease such as an STD. It is believed that in finding the connexion between expression or activity profile of the enzyme and formation of infectious pathogen, the inventors have in a sense discovered the earliest molecular symptom of disease re-activation or formation of active disease. It is for this reason that the invention finds particular application to individuals

who are otherwise asymptomatic for symptoms of disease, such as asymptomatic HSV-2-positive individuals.

Typically the enzyme has substrate specificity for tryptophan or for arginine. In one particularly preferred embodiment the enzyme is human indoleamine 2,3-dioxygenase (IDO). In particular as described herein it has been found that IDO has increased expression or activity in the vaginal mucous of an individual infected with HSV-2.

In another embodiment, the enzyme may be NOS2, otherwise known as inducible nitric oxide synthase.

In one particular embodiment, the enzyme may have substrate specificity for other than an amino acid. One example of an enzyme within this embodiment is myeloperoxidase (MPO) and the relevant assay determines for whether the expression or activity of MPO is enhanced.

The method of the invention may involve measuring the expression or activity of the relevant enzyme, or both expression and activity may be measured. In one embodiment, the invention includes:

determining whether the individual contains an increased amount of the enzyme;

to determine whether the individual contains an increased expression of the enzyme. '*Expression of the enzyme*' generally refers to the amount of enzyme produced in the individual or sample. Generally this is a measure of the amount of enzyme in the individual or sample at the time of the application of the method, although in some embodiments, it may also be possible to alternatively, or additionally measure the rate of expression of the enzyme.

The amount of enzyme in the relevant sample can be determined by methods that directly detect the level of protein enzyme, for example by serology are preferred, although where there is an understood linkage as between the amount of enzyme protein and amount of polynucleotide, for example RNA, it may be possible to additionally or alternatively determine the amount of RNA from which the enzyme is translated. Examples of these methods are further described below.

In certain embodiments, the invention includes the steps of:

determining whether the individual contains an increased amount of a catabolite of the enzyme;

thereby determining whether the individual contains an increased activity of the enzyme.

A '*catabolite*' is generally understood as being a molecular species arising from reaction of an enzyme with substrate. The catabolite may be an '*immediate species*' i.e. a species arising immediately from the reaction of enzyme with substrate. In certain embodiments, the catabolite may be another molecular species arising from metabolism or other modification of the immediate species. As an example, where the enzyme is IDO and the substrate is L-tryptophan, the immediate species is N-formylkynurenine. Other molecular species arising from metabolism of N-formylkynurenine that could be measured or detected are formic acid or kynurenine.

In another example, specifically where the enzyme is NOS2, the catabolite may be nitrite or related nitrite ion. Nitrite-indicator polymer matrices for identifying nitrites in urine are well known in the art.

In a preferred embodiment, for example where the intention is to determine whether an individual contains infectious HSV-2 pathogen, the method includes the following steps:

determining whether the individual contains an increased amount of kynurenine;

thereby determining whether the individual contains an increased activity of the enzyme.

In another embodiment, the method includes the step of determining whether the individual contains an increased amount of a molecule, the expression of which is induced on exposure to a catabolite of IDO. One example is the aryl hydrocarbon receptor (AHR), which binds to kynurenine.

In certain embodiments, the method includes the follow steps:

providing a sample of body fluid obtained from the individual and thereafter;

determining whether the sample contains an increased expression or activity of an enzyme of the individual that has substrate specificity for an amino acid;

wherein the individual is determined to contain an infectious pathogen where the sample is determined to contain an increased expression or activity of the enzyme. In these embodiments the sample of body fluid is made available before commencement of the method of the invention. The skilled worker is aware of many methods for obtaining a sample of body fluid. One particularly preferred method, particularly where the infectious pathogen or contagious disease of interest is HSV-2 and genital herpes respectively, is that which produces a sample of vaginal mucus. This method might include scraping or wiping inside the vagina to obtain a sample.

As described herein, in certain embodiments, the method includes the step of providing a sample of body fluid. Any body fluid that is expected to contain infectious pathogen and that is transmissible from one individual to another as a consequence of contact of skin or mucous membranes could be used in a method described herein. Examples include blood, plasma, serum, lymph, semen, saliva, sputum, sebum, uterine and vaginal secretions and related mucous and tear. The body fluid is typically cell-free although in some circumstances it may contain residual cells or fragments thereof. In a particularly preferred embodiment, the sample contains vaginal mucus.

The methods described herein are applicable to the assessment of whether an individual, particularly an asymptomatic individual is at risk for spreading a sexually transmissible disease. As described, the individual may be aware of whether he/she has an infection with a relevant pathogen, but unaware, for example, prior to intercourse, as to whether he/she is likely to contain infectious pathogen capable of being transmitted during intercourse to a partner, thereby establishing disease in the latter.

Examples of particular diseases of interest include genital herpes, Chlamydia, Gonorrhoea, *Syphilis* and *Candidiasis*. In a particularly preferred embodiment, the STD is herpes.

The infectious pathogen the subject of the methods described herein may be a virus, bacteria, fungus or other micro-organism. Particularly examples include HSV-1, HSV-2, HIV, HPV.. In a particularly preferred embodiment, the infectious pathogen is HSV-2.

A variety of techniques are available to the skilled addressee for the detecting or measuring expression, production or amount of enzymes and relevant catabolites. A general discussion follows.

The presence of a given protein, or level of expression of a given protein in a sample, such as an enzyme or protein or protein catabolite thereof can be detected by any number of assays. Examples include immunoassays, chromatography and mass spectrometry.

Immunoassays, i.e. assays involving an element of the immune system are particularly preferred. These assays may generally be classified into one of:

(i) assays in which purified antigen is used to detect an antibody in host serum. For example, purified antigen is bound to solid phase by adsorption or indirectly through another molecule and host serum or other body fluid is applied followed by another antibody for detecting presence or absence of host antibody;

(ii) assays in which purified antibody specific for antigen is used to detect antigen in the host. For example, purified antibody is bound to solid phase, host body fluid is then applied followed by another antibody specific for the antigen to be detected. There are many examples of this approach including ELISA, RIA and the like;

(iii) assays in which body fluid is separated from a protein component contained within it, the protein component is then fixed onto a solid phase and the probed with an antibody. Examples include dot blotting and Western blotting.

Thus in one embodiment the method is implemented as a direct, indirect or sandwich ELISA, RIA or like assay involving the application of a liquid sample to an assay system. The sample may or may not be processed prior to contact with an antibody.

Where the expression or activity of the enzyme is to be detected or measured by direct detection or measurement of a catabolite that is not a protein, an immunoassay format may not be appropriate. A further assay format which does not require formation of an immune complex is one in which an assay output is the result of catalysis of a substrate and the output is observed for example by measuring a change in optical density.

Examples of antibodies that are useful in the invention are those that bind selectively or specifically to IDO, NOS2 or to catabolites of these enzymes. Where the catabolite is a small molecule, it may be conjugated to a hapten to increase molecular weight, thereby enabling an appropriate humoral response for antibody production. The antiserum may be monoclonal or polyclonal.

Aside from antibodies, other capture agents include those which bind to a catabolite of IDO, NOS2. In one example the capture agent is a receptor. For example, where the catabolite is kynurenine, the capture agent may be the AHR receptor. In another example, the capture agent is an enzyme. For example, where the catabolite is 3 kynurenine, the capture agent may be kynurenine-3-hydroxylase.

Other assay formats may involve various types of chromatography. Examples include chromatographic methods that are not based on serology. In one example, the chromatographic method may employ a colour change to indicate the presence or absence of infectious agent or contagious disease.

In another embodiment, the assay format may involve a particular chemical reaction, such as an Ehrlich reaction, and the assay detects for the presence or absence of the relevant reaction product.

As described above, in one embodiment, a nucleic acid that encodes the enzyme, or that is complementary to a nucleic acid that encodes the enzyme, is measured. In this embodiment, the nucleic acid may be one which can be used to determine the presence of a given protein, or level of expression of a given protein in a host as per a molecular genetic approach. One example is where a polynucleotide that is complementary to a nucleic acid (DNA, RNA, cDNA) that encodes a target protein is hybridised to the nucleic acid and hybridisation is detected. One example is quantitative PCR. Others include quantitative Northern and Southern blotting, and microarray. As is well known in the art, hybridisation of nucleic acid molecules may be controlled by the type of buffer used for hybridisation and the temperature of the buffer. '*High stringency conditions*' are conditions in which the buffer includes about 0.1 x SSC, 0.1% SDS and the temperature is about 60^0C.

Typically the enzyme or catabolite is directly detected. This is otherwise known as a '*direct detection*' of the enzyme or catabolite to measure the level of expression or activity of the enzyme. This may involve detection of the whole enzyme or fragment of it. In certain embodiments, the level of expression of a molecule, the expression of which is modulated in accordance with the up-regulation of expression or activity of the enzyme is measured. This is otherwise known as an '*indirect detection*' of the enzyme to measure the level of expression or activity of the enzyme.

In embodiment, it may be necessary to compare the expression or activity of the enzyme with an individual who is known not to be infected with the pathogen. The latter ostensibly serves as a negative control or uninfected control. Thus in one embodiment, the method includes the step of:

comparing the expression or activity of the enzyme of the individual with the expression or activity of the enzyme of an individual that does not contain the pathogen (the latter being a negative control),

thereby determining whether the individual contains an increased expression or activity of the enzyme.

Typically the level of expression or activity of the relevant enzyme in the individual is assessed by the steps of:

measuring the level of expression or activity of the enzyme; and

comparing the measured level with an uninfected control that describes the level of expression or activity of the enzyme as observed in the same tissue of a subject that has been determined as not having an infection with the relevant pathogen.

An '*uninfected control*' may be derived by measuring the level of expression or activity of the enzyme in a tissue or fluid of a subject that, but for an absence of a relevant infection, is generally the same or very similar to the tissue or fluid of the individual selected for assessment for likelihood of infectious pathogen (the latter otherwise known as the '*test sample*').

In certain embodiments, the uninfected control is derived by measuring the level of expression or activity of the enzyme in a cell line or an engineered tissue.

In certain embodiments, the method includes measuring the level of expression or activity of the enzyme in the uninfected control to compare the measured level in the test sample with the level in the uninfected control.

In other embodiments, an internal standard is applied. This may be used to ensure that the method operates within accepted decision limit quality control criteria. The assay then provides a value/number/result or other output for each test sample. That output is then deemed to represent infection or non-infection based on independent data derived from frequency distributions of results from an uninfected control. This control may describe distributions of results obtained from more than one uninfected subject, for example, from an uninfected population which may be of the same species, geographic origin, age, sex as the test sample. A positive-negative cut-point for the method is determined from these distributions to provide defined levels of diagnostic sensitivity and specificity for the method.

The internal standard or reference may obviate the need to physically provide an uninfected control in the form of uninfected cells or fluid or otherwise to physically measure the level of the target in an uninfected control. Where the internal standard or reference is used, the level of expression in an uninfected control has been predetermined and may be provided for example in the form of written information that is supplied with a diagnostic kit.

The measurement of the level of expression or activity of the enzyme in the fluid or tissue of the subject for deriving the uninfected control is generally done using the same assay format as that that is used for measurement of the target in the test sample. However, it is not necessary to use the same assay when an internal standard that can be used to compare data obtained from different assay formats is or has been applied.

In another embodiment, the level of expression or activity of the enzyme in the individual is assessed by the steps of:

measuring the level of expression or activity of the enzyme in a fluid or tissue of the individual; and

comparing the measured level with an infected control that describes the level of expression or activity of the enzyme as observed in the same tissue of a subject that has been determined as having infection with the relevant pathogen.

An '*infected control*' is generally derived by measuring the level of expression or activity of the enzyme in a fluid or tissue of a subject that is generally the same or very similar to the fluid or tissue of the test sample. The measurement of the level of expression or activity of the enzyme in the fluid or tissue of the subject for deriving the infected control is generally done using the same assay format that is used for measurement of the target protein in the test sample.

In certain embodiments, the method includes measuring the level of expression or activity of the enzyme in the infected control to compare the measured level in the test sample with the level in the infected control. However, again an internal standard may be applied that obviates the need to provide an infected control or otherwise to measure the level of the target in an infected control.

The measurement of the level of expression or activity of the enzyme in the fluid or tissue of the subject for deriving the infected control is generally done using the same assay format as that that is used for measurement of the enzyme in the test sample. However, again it is not necessary to use the same assay when an internal standard that can be used to compare data obtained from different assay formats is or has been applied.

In accordance with the above, in one embodiment, the method includes:

measuring the level of expression or activity of the enzyme in a fluid or tissue of the individual; and

comparing the measured level with an uninfected control and an infected control.

The amount of enzyme may be variously described in terms of specific units of activity, weight per volume or weight of sample, or moles per volume, depending on the method used for measurement or detection of amount of enzyme.

In one particularly useful embodiment, the assay format further includes a means for detecting for the presence of a relevant pathogen. The purpose of this means is to determine the

reason underlying the increased expression or activity of the relevant enzyme. Accordingly, the assay format identifies whether there is a pathogen in an activated state, or a disease in contagious form and identifies the relevant pathogen. In one embodiment, the assay format includes means for detecting for the presence of HSV-2. In another embodiment, the assay format includes means for detecting the presence of more than one pathogen. For example, the assay may include single means for detecting more than one pathogen, or multiple means each for detecting a specific pathogen only.

Examples

We have discovered that mice infected intravaginally with HSV-2 express robust levels of indoleamine 2,3-dioxygenase (IDO) in vaginal epithelial cells. IDO is an enzyme that catabolises the essential amino acid L-tryptophan (L-Trp) to N-formylkynurenine, which is then converted to formic acid and the stable end-product kynurenine (Kyn).

In HSV-2-infected mice, the Kyn produced by IDO^+ vaginal epithelial cells was secreted into the lumen, and thus, Kyn was detectable in the vaginal mucus by high performance liquid chromatography (HPLC) (Figure 1 and 2). Importantly, despite the presence of commensal organisms in the vaginal tract, these microorganisms do not elicit any detectable IDO in the mouse vagina (mRNA, protein or activity).

We propose that the presence of Kyn in the mucus is a novel diagnostic marker of recrudescent HSV-2 infection in humans, since the expression of IDO after the first infection is even higher upon secondary exposure.

The standard HSV-2 mouse model involved subcutaneous injection of 4 mg medroxyprogesterone acetate 7 days prior to infection. In addition to synchronising all mice to the same progesterone-dominant phase of the reproductive cycle, this renders the mice uniformly susceptible to intravaginal infection.

After 7 days, mice were anaesthesised via intraperitoneal injection of Avertin. The vagina was washed three times with 50 µL sterile phosphate-buffered saline using a flame-polished bevelled-edge 200 µL plastic pipette tip to remove excess mucus. HSV-2 (strain 183; 10^5 plaque-

forming units) was then introduced intravaginally using a sterile 200 µL pipette tip in a final volume of 20 µL. Mock-infected mice were inoculated with 20 µL sterile phosphate-buffered saline (vehicle). To ensure the inoculum did not leak out of the vagina, the mice were laid supine with their pelvis raised for at least 30 min before transfer into their respective cages.

On day 1, 3, 5, 7 and 10 post-infection, mice were euthanised and the vaginal lumen was washed 3 times with 50 µL phosphate-buffered saline to collect the mucus. The mucus was stored at -80°C until analysis.

To prepare the samples for analysis, the vaginal wash samples were thawed and mixed with 20% (w/v) ice-cold trichloroacetic acid in a 3:1 ratio and centrifuged at 16100 x *g* for 15 min at 4°C. The clarified supernatant was stored at -80°C until analysis by HPLC using a Hypersil 3 µm ODS C18 column. Samples were eluted with 100 mM chloroacetic acid and 9% acetonitrile (pH 2.4) at a rate of 0.5 mL/min. L-Trp and Kyn were detected by UV absorbance at 280 and 364 nm, with retention times of ~10-12 min and 6-7 min, respectively. To determine the concentration of L-Trp and Kyn in the mucus, peak areas were compared to authentic L-Trp and Kyn standards of known concentrations that are included in each run.

CLAIMS

1. A method for determining whether an individual has an infectious pathogen including:

determining whether the individual contains an increased expression or activity of an enzyme of the individual that has substrate specificity for an amino acid;

wherein the individual is determined to have an infectious pathogen where the individual is determined to contain an increased expression or activity of the enzyme.

2. The method of claim 1 wherein the individual does not have a symptom that is associated with infection of an individual with the pathogen.

3. The method of claim 1 or 2 wherein the pathogen is contained in the individual in an amount that is insufficient to enable direct detection of the pathogen in the individual.

4. The method of any one of the preceding claims wherein the enzyme has substrate specificity for an essential amino acid.

5. The method of any one of the preceding claims where the enzyme has substrate specificity for tryptophan or arginine.

6. The method of any one of the preceding claims wherein the enzyme is indoleamine 2,3-dioxygenase (IDO).

7. The method of any one of the preceding claims wherein the infectious pathogen is an aetiological agent of a sexually transmissible disease.

8. The method of any one of the preceding claims wherein the infectious pathogen is a virus.

9. The method of any one of the preceding claims wherein the virus is HSV-2.

10. The method of any one of the preceding claims including the step of:

comparing the expression or activity of the enzyme of the individual with the expression or activity of the enzyme of an individual that does not contain the pathogen,

thereby determining whether the individual contains an increased expression or activity of the enzyme.

11. The method of any one of the preceding claims including the step of:

determining whether the individual contains an increased amount of the enzyme;

thereby determining whether the individual contains an increased expression of the enzyme.

12. The method of any one of the preceding claims including the step of:

determining whether the individual contains an increased amount of a catabolite of the enzyme;

thereby determining whether the individual contains an increased activity of the enzyme.

13. The method of any one of the preceding claims including the step of:

determining whether the individual contains an increased amount of kynurenine;

thereby determining whether the individual contains an increased activity of the enzyme.

14. The method of any one of the preceding claims including the step of:

providing a sample of body fluid obtained from the individual and thereafter;

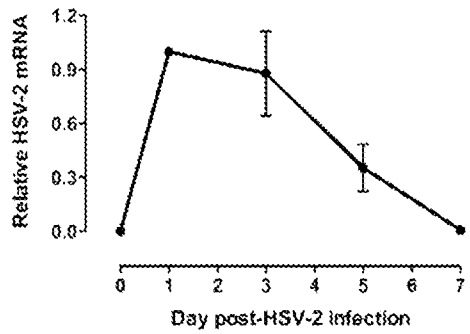
determining whether the sample contains an increased expression or activity of an enzyme of the individual that has substrate specificity for an amino acid;

wherein the individual is determined to contain an infectious pathogen where the sample is determined to contain an increased expression or activity of the enzyme.

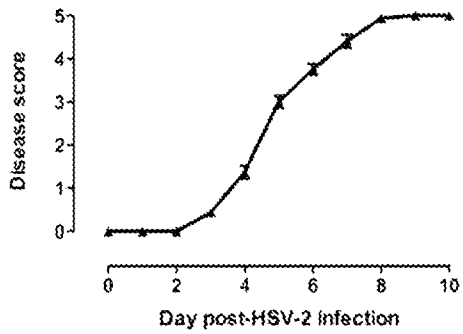
15. The method of claim 14 wherein the sample contains vaginal mucus.

Figure 1

(A)



(B)



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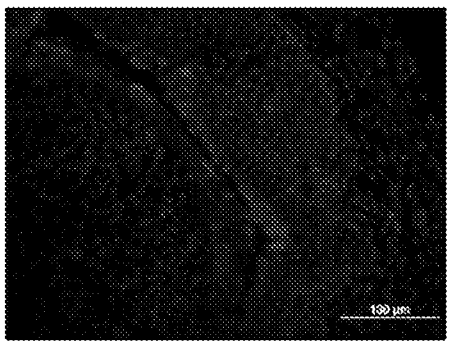

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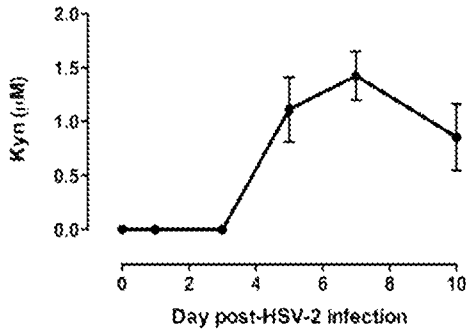

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2014/000254

A. CLASSIFICATION OF SUBJECT MATTER

C12Q 1/26 (2006.01) C12Q 1/68 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, Epodoc, Medline, HCAplus, Biosis, Embase, search terms: HSV, pathogen, sexually transmitted, amino acid, IDO, kynurenine, assay, nitric oxide synthase, inducible, viral and like terms

Inventor/Author search on Patentscope and Google Scholar: Nicholas King, Shane Thomas, Amanda Yeung

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

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"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"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

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Date of the actual completion of the international search	Date of mailing of the international search report
11 June 2014	11 June 2014
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Brook Clinton AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262837951

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/AU2014/000254**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2006/0099628 A1 (CHING et al.) 11 May 2006 abstract, claims, claim 3, claim 6, paragraphs 10, 16	1-6, 10, 14
X	RAPOPORT, M. I. and BEISEL, W. R. 'Studies of tryptophan metabolism in experimental animals and man during infectious illness'. The American Journal of Clinical Nutrition, 1971, vol. 24, no. 7, pages 807-814. pg. 809, Fig. 3, 6	1, 4, 5, 10-14
X	BECERRA, A., et al. 'Increased activity of indoleamine 2,3-dioxygenase in serum from acutely infected dengue patients linked to gamma interferon antiviral function'. Journal of General Virology, 2009, vol. 90, pages 810-817. Fig. 2, pg. 813	1, 4-6, 8, 10-14
X	REINHARD, J. F. 'Altered tryptophan metabolism in mice with herpes simplex virus encephalitis: increases in spinal cord quinolinic acid'. Neurochemical Research, 1998, vol. 23, no. 5, pages 661-665 Table 1	1, 4-8, 10-13
X	Sanni, L. A., et al. 'Dramatic changes in oxidative tryptophan metabolism along the kynurenine pathway in experimental cerebral and noncerebral malaria'. American Journal of Pathology, 1998, vol. 152, no. 2, pages 611-619. Table 2.	1, 4-6, 10-13
X	WO 1997/034014 A1 (YALE UNIVERSITY) 18 September 1997 page 1, lines 10-15; page 4, line 22 to page 5, line 8; page 5, lines 22-24; page 8, lines 16-19; page 9, lines 2-9	1, 4, 5, 10-12, 14, 15
P,X	Azardaryany, M. K., et al. 'Modulation of immunity against gential Herpes simplex virus'. Cytokine, September 2013, vol. 63, page 245, abstract 11 whole document	1, 4-9, 12, 13

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2014/000254**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
US 2006/0099628 A1	11 May 2006	WO 2007018574 A2	15 Feb 2007
WO 1997/034014 A1	18 September 1997	AU 2990497 A	01 Oct 1997
		CA 2248558 A1	18 Sep 1997
		EP 0907747 A1	14 Apr 1999
		US 2002164658 A1	07 Nov 2002
		US 6605447 B2	12 Aug 2003

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.