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METHOD FOR THE DETECTION OF VIRUS

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10 Claims

ABSTRACT OF THE DISCLOSURE

The presence or absence of virus may be detected by inoculating a tissue culture, which is an appropriate host for the suspected virus, with a sample of the material suspected to contain a virus and then comparing the amount of adenosine triphosphate in the culture before and after a period of incubation. The adenosine triphosphate content of the inoculated tissue culture is determined by reacting the tissue culture with a mixture comprising luciferin, luciferase, and a cation in the presence of oxygen and monitoring the amount of bioluminescence given off thereby. A significant change in the amount of bioluminescence after incubation indicates the presence of virus, whereas the absence of virus is indicated by no significant change.

CROSS REFERENCES TO OTHER APPLICATION

This application is a continuation-in-part of copending application Ser. No. 739,913 filed June 18, 1968, which in turn is a streamlined continuation application of application Ser. No. 433,462 filed Feb. 17, 1965 and copending therewith both now abandoned.

This invention relates to a method for the detection of virus. More particularly, this invention relates to the utilization of the bioluminescent reaction between adenosine triphosphate (hereinafter referred to as ATP) and firefly lantern extract as a means for detecting the presence of an invading virus in a host tissue.

It is known that ATP is present in all living cells and tissues. The energy requirements for all biological reactions are directly or indirectly supplied through ATP. It has been found that any change in cellular integrity, such as the invasion of a virus, will be reflected in the kinetics of ATP metabolism.

It is an object of this invention to provide a method for the rapid detection of virus in a material by monitoring the ATP level of the material.

It is another object of this invention to utilize the bioluminescent reaction between ATP and firefly lantern extract as a means for detecting the presence or absence of a virus in a host cell.

These and other objects are attained by the practice of this invention which, briefly, comprises inoculating a tissue culture, which is an appropriate host for the suspected virus, with a sample of the material suspected to contain a virus. The ATP content of the inoculated tissue culture is monitored both before and following an incubation period. The presence of a virus in the material being tested will be indicated by a significant change (e.g. increase or decrease) in the ATP content of the inoculated tissue culture following the period of incubation since it has been found that the ATP content of virus-containing cells is different than the amount of ATP in normal cells.

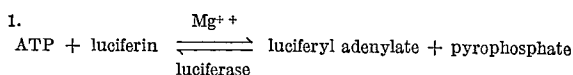
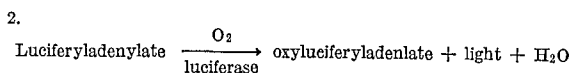
In the preferred embodiment of this invention, duplicate tissue cultures are provided and one of the tissue cultures is inoculated with a sample of the unknown material which is suspected to contain a virus. After a suitable incubation period, equal aliquots of each of the tissue

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cultures are mixed in the presence of oxygen with firefly lantern extract which includes a mixture comprising luciferin, luciferase and a cation such as magnesium. A reaction will occur which is accompanied by the emission of light. The quantity of light is measured and the amount of light emitted by the inoculated tissue culture is compared with the amount of light emitted by the non-inoculated tissue culture. The presence of a virus in the unknown material will be indicated by a significant change in the amount of light emitted by the inoculated tissue culture over the non-inoculated tissue culture on an equal aliquot basis while the absence of virus will be indicated by no significant change. The emission of light is caused by the reaction of ATP with the constituents of the firefly lantern extract in the presence of oxygen. Since the ATP content of virus-containing cells is significantly different than the amount of ATP found in normal cells, the virus-containing cells will emit a different amount of light when mixed with firefly lantern extract than normal cells. Thus, the practice of this embodiment of the invention utilizes the phenomenon of firefly bioluminescence to establish the presence of a virus by the reaction of ATP with firefly lantern extract.

The practice of this invention may also be accomplished by inoculating a tissue culture with a sample of the material which is suspected to contain a virus, immediately assaying an aliquot of the tissue culture for ATP content, and assaying an equal aliquot of the tissue culture for ATP content following a suitable incubation period. A significant change in ATP content following incubation, on an equal aliquot basis, will indicate the presence of a virus in the unknown material. The ATP assay may be conducted by the firefly bioluminescent method previously referred to.

The reactants required for firefly bioluminescence are the substrate, luciferin; the enzyme, luciferase; the activator, ATP; a cation (usually magnesium); and oxygen. The overall reaction is an oxidation reaction catalyzed by the enzyme, luciferase, which results in the emission of light. A general mechanism of the course of the reaction is as follows:



This reaction is absolutely specific for ATP. The ATP may not be replaced by any other known compound.

The firefly bioluminescent reaction may be carried out utilizing crude firefly lantern extracts or the purified constituents therefrom which participate in the bioluminescent reaction. It has been found that a sufficiently high degree of sensitivity may be attained using the primary extract of the firefly lantern.

Lyophilized firefly lantern extract may be obtained commercially. This material may be prepared for use by dissolving it in distilled, deionized water to the desired concentrations. The extract used in the examples which follow, unless otherwise specified, were obtained by dissolving 70 mg. of lyophilized firefly lantern extract in 5 ml. of water. The lyophilized preparation also contains MgSO_4 and potassium arsenate in amounts sufficient to result in concentrations of 0.01 M and 0.05 M, respectively. The pH of such a solution is 7.4. The solutions may be further diluted to give any desired concentration of firefly lantern extract.

The firefly lantern extract which may be used in the practice of this invention may also be prepared in the

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laboratory from desiccated firefly tails. The firefly tails are first ground to a fine powder with a mortar and pestle with a small amount of washed silica. The powder is then extracted with 0.05 M potassium arsenate-0.01 M MgSO_4 at pH 7.4.

When crude firefly lantern extract is used in the practice of this invention, there may be present in the extract small amounts of ATP and ATP precursors, along with phosphorylating enzymes capable of converting the precursors into ATP. The presence of these materials may give rise to a basal light emission by the firefly extract in the absence of exogenous ATP. This type of light emission, which is referred to an inherent light, occasionally may interfere with the detection of light emission in the practice of this invention. However, the problem of inherent light may be eliminated or minimized by one or more of the following techniques:

(1) The firefly extract may be partially purified to remove the factors responsible for the inherent light. The separation and partial purification of luciferase and luciferin is described by McElroy (Methods in Enzymology, vol. II, page 851, Academic Press, Inc., New York, 1955).

(2) Another approach to the removal of inherent light involves the "salting out" of luciferase by the addition of ammonium sulfate to the firefly extract leaving the non-protein factors responsible for inherent light in the supernatant. This has been accomplished as follows: 50 mg. of lyophilized extract were suspended in 10 ml. of 2.7 M ammonium sulfate. After standing at room temperature for 15 minutes, the suspension was centrifuged at approximately 200G for 10 minutes after which the supernatant was discarded. The precipitate, after being washed twice with 10 ml. aliquots of 2.7 M ammonium sulfate, was taken up in 2.5 ml. of a solution of 0.05 M potassium arsenate buffer (pH 7.4) and 0.01 M magnesium sulfate. This treatment, followed by reconstitution with partially purified luciferin, reduces the overall activity of the extract by only about 15 percent and reduces the inherent light by about 90 percent.

(3) Experiments have indicated that the use of calcium phosphate gel will also reduce the inherent light in the extract without significantly reducing the activity of the extract. Thus, 50 mg. of commercial lyophilized firefly extract were dissolved in 1.25 ml. of deionized water and centrifuged. The solution was then treated from one to three times with varying amounts of calcium phosphate gel (from 249 mg. to 334 mg.). The treatment consisted of shaking the gel with the extract for ten minutes and then removing the gel by centrifugation at 200G for 10 minutes. With one treatment with calcium phosphate gel, there is a reduction of about 85 percent of the inherent light with only a loss of from 7 to 26 percent of luciferin-luciferase activity.

(4) The simplest means for reducing the inherent light is by dilution of the extract with water. Maximum sensitivity with the least amount of inherent light is obtained at a lyophilized extract concentration of 3 mg./ml.

The practice of this invention may be used to determine rapidly the presence of a suspected virus present in small quantities of tissues. Using electronic equipment, tissues or cells containing an amount of ATP less than 2×10^{-4} $\mu\text{g.}$ and approaching 10^{-7} $\mu\text{g.}$, may be assayed. The bioluminescent reaction itself takes less than 0.5 second to attain maximum amplitude.

The tissue cultures which may be used in the practice of this invention are commercially available. The particular tissue cultures used should be appropriate hosts for the virus which is suspected to be present in the unknown material.

Listed below are several pathogenic viruses characterized by their severe infectious nature and/or their frequent occurrence. Also listed are appropriate host tissue cultures for each virus,

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Virus		Tissue culture	
Eastern equine encephalomyelitis		HeLa cells, chicken embryo.	
Venezuelan equine encephalomyelitis		L cells (mouse).	
Western equine encephalomyelitis		Hamster kidney.	
5 St. Louis encephalitis		Chicken and mouse embryo.	
Yellow fever		Chicken and mouse embryo.	
Rabies		Hamster kidney, mouse	
		ependymoma.	
Poliomyelitis		Monkey kidney.	
Influenza		Chicken embryo.	
Adenovirus		HeLa cells, monkey kidney.	
10 Variola (small pox)		HeLa cells, chick embryo.	
Psittacosis		HeLa cells, monkey kidney.	
Herpes B		Monkey kidney.	
Coxsackie A and B		Monkey kidney.	
ECHO		Monkey kidney, human	
		amniion.	

The tissue culture which is an appropriate host for the suspected virus is divided into equal aliquots to provide duplicate cultures of the tissue. One of the cultures is then inoculated with a sample of the unknown material. Both the inoculated and the non-inoculated cultures are then incubated under conditions standard for the particular tissue cultures used. After a suitable period of incubation, the time of which will vary with the particular virus being tested for, each tissue culture is assayed for ATP content by contacting a sample of it, in the presence of oxygen, with firefly lantern extract and measuring the amount of light emitted. Preferably, the tissue cultures are assayed at periodic intervals after one has been inoculated with the unknown material. Comparisons of ATP content are made on the basis of ATP per unit tissue volume or ATP per unit protein weight or both.

The practice of this invention may be used to detect the presence of a virus in materials such as filtered air, drinking water, tissue samples, throat swabbings, excretions from lesions, etc. Samples of such materials may be obtained in conventional fashion.

When a material is suspected to contain a virus but it is not known what type of virus may be present, the practice of this invention may be used not only to confirm the presence of a virus but also to indicate the type of virus. This may be accomplished by inoculating several different types of tissue cultures, each of which is a host for a different group of viruses. Each of the types of tissue cultures is then inoculated with the material suspected to contain a virus, incubated and assayed as previously described. If the results show that one of the types of tissue cultures has undergone a significant change in ATP content, the presence of a virus will be confirmed and it will be established that the virus is one of the group for which that particular tissue culture is a host.

The specific type of virus may be determined by providing equal aliquots of the host tissue culture and adding a different anti-serum, each of which is specific for a different virus, to each aliquot. Each aliquot is then inoculated with the material containing a virus. Following a suitable incubation period, the aliquots are assayed for ATP content as previously described. If the results show that one tissue culture has not undergone a significant change in ATP content, the unknown virus will be identified as the one for which the anti-serum contained in that tissue culture is specific.

In assaying the tissue cultures, results may be obtained by mixing the intact tissue cells with the firefly lantern extract. However, for maximal response and in order to make more accurate assays, it is preferred to rupture the cells and extract the ATP therefrom. A variety of methods for the extraction of ATP from the cells may be used. These include hot water extraction, acetone extraction, ultrasonic disruption, dimethylsulfoxide extraction and perchloric acid extraction. Some of the methods which may be used for accomplishing rupture and extraction of the cells are described below:

(A) Acetone and hot water: One ml. of the tissue culture is added to 10 ml. of deionized water and the suspension maintained at 100°C. for one to five minutes. The preparation is then cooled and assayed for ATP.

The acetone extraction consists of adding one ml. of a washed tissue culture suspension to ten ml. of acetone. After standing for one to five minutes, a one ml. aliquot of the mixture is taken to dryness in air and the residue suspended in one ml. of deionized water. The preparation is then assayed.

(B) Dimethyl sulfoxide (DMSO): DMSO is a high-boiling point organic solvent, miscible with water in all proportions and exhibiting a very low order of toxicity. One ml. aliquots of the tissue cell suspension are added to ten ml. of various concentrations of DMSO in water. After standing for five minutes, the suspension is assayed for ATP response.

(C) Ultrasonic oscillation: Ultrasonic oscillation has been successfully used by many investigators for the rupture of cells. Five ml. of tissue cell suspension are subjected to one to five minutes of ultrasonic oscillation at approximately 57-75 watts. After cooling the tube in flowing water, the treated suspension is assayed for ATP response. Trichloroacetic acid (0.5 ml. of 5 percent solution) may be added to the cell suspension prior to sonification in order to stabilize the ATP against hydrolysis.

(D) Perchloric acid: Up to 0.2 ml. of perchloric acid may be added to 5 ml. of tissue cell suspension. The preparation is then assayed.

It is preferred to contact the tissue culture to be assayed and the firefly lantern extract in a liquid reaction medium. The liquid reaction medium will generally contain enough dissolved oxygen to allow the bioluminescent reaction to take place.

The material to be assayed should be mixed with the firefly lantern extract in a manner which permits the mechanical measurement and recordation of the light emitted.

The procedure for using the instruments which are used to detect and record the intensity of emitted light consists of injecting a liquid medium containing the material to be assayed, such as an aqueous extract of the material, into a cuvette containing the firefly lantern extract. The extract is held at pH 7.4 with potassium arsenate buffer. The light emitted as the result of the reaction between the ATP in the tissue culture to be tested and the firefly lantern extract strikes the surface of a photomultiplier tube giving rise to a current which can be measured and recorded by either an oscilloscope photograph or a linear recorder. The unit of intensity used for comparing these reactions is the millivolt. Alternately, a pulse counting device with a digital or analogue read-out may be used to record the reaction.

Because the response (i.e., light emission) is almost instantaneous when the tissue culture is contacted with the firefly lantern extract, the extract should be positioned in front of the light detection system prior to the introduction of the material to be assayed.

There are two ways in which the bioluminescent response with ATP present in a material can be expressed. One is by measurement of the maximum intensity of the emitted light, which after reaching this maximum value, decays exponentially. With all other factors constant, the maximum intensity is directly proportional to the concentration of ATP. The alternative manner of expressing the response is by integration of the total amount of light emitted; i.e., area under the light intensity curve. This is the slower of the two methods, because of the relatively long time necessary for complete decay (up to 10 minutes). Therefore, maximum intensity has been chosen as the measure of ATP response.

The instrumentation necessary for the quantitative measurement of bioluminescence consists of a photomultiplier tube for the conversion of light energy into an electrical signal, a device for determining the magnitude of the signal, and a light-tight chamber for presentation of the bioluminescent reaction to the photomultiplier tube.

In one system, part of the assembly consists of a composite sensing and reaction chamber which contains a photomultiplier tube, with appropriate circuitry, and a ro-

tary cylinder mounted in a block of aluminum in a manner which permits removal of the reaction chamber without exposing the phototube to light. A section of the cylinder wall is cut out to accommodate a standard ten mm. or five mm. rectangular cuvette. Immediately above the cuvette holder is a small injection port sealed with a replaceable light-tight rubber plug. The entire unit is painted black to reduce light reflection. The photomultiplier converts the light energy into an electrical signal. An oscilloscope which records the magnitude of the signal from the photomultiplier, is provided with a maximum sensitivity of 200 u.v./cm. of beam deflection which will allow an increase in system sensitivity by decreasing the bandwidth or directly reducing the noise level. There is a multiple switching arrangement at the scope input which makes it convenient to adjust the system zeros and balances. The differential input to the scope provides a means to balance the dark current output of the phototube. The response to the firefly luminescent system displayed on the oscilloscope screen is recorded with a camera which mounts directly onto the front of the oscilloscope. To observe and record the reaction, the cuvette containing the necessary reagents is positioned in the cuvette carrier without exposing the phototube. Rotation of the carrier positions the cuvette in front of the phototube. The tissue culture is then added through the injection port and the magnitude of the response is recorded by the camera.

A typical procedure for assaying a material according to the practice of this invent on utilizing electronic apparatus to detect and record the intensity of the bioluminescent reaction is described below:

PROCEDURE A

One ml. of a 0.5 percent buffered aqueous solution of commercially available lyophilized firefly lantern extract is placed into a cuvette which is then positioned in the light detection chamber of the type previously described. The extract contains luciferase, luciferin and magnesium. Sufficient dissolved oxygen for the bioluminescent reaction is present in the solution. A portion of the tissue culture to be assayed is subjected to ultrasonic vibration for one or more minutes. One tenth ml. of the suspension is then drawn into a hypodermic syringe and immediately injected through the light-proof seal into the cuvette. The reaction reaches maximum light intensity in less than 0.5 second and then decreases exponentially for several minutes. The entire procedure can be executed and recorded in less than two minutes.

In order to make quantitative determinations of the amount of ATP present, the instrument used to measure the light response may be calibrated using known concentrations of ATP. A calibration may be plotted by injecting 1/10 ml. portions of known concentrations of ATP through the light-proof seal into the cuvette by means of a hypodermic syringe. The light response in millivolts is plotted against the ATP concentration. A straight linear function is obtained. For example, if the response from 10^{-1} grams of ATP is 20,000 millivolts, that from 10^{-2} is 2000 millivolts, etc.

The overall sensitivity and perhaps reliability of the bioluminescent reaction of the material to be tested may be increased by the conversion of other nucleotide phosphates which are present in tissues, such as adenosine diphosphate (ADP) and adenosine monophosphate (AMP), to ATP. This may be accomplished as described below by adding to the tissue culture certain phosphorylating enzymes. One such enzyme is phosphocreatine kinase.

Phosphocreatine kinase (10-30 units/mg.) is prepared in a concentration of 0.4 mg./ml. in 0.05 M potassium arsenate buffer (pH 7.4) containing MgSO_4 at a concentration of 10^{-5} and creatine phosphate at a concentration of 0.1 mg./ml. One tenth ml. of this solution is added to 1 ml. of a solution being assayed for ADP. The mixture is allowed to incubate at 30° C. for 30

minutes. The mixture is then boiled for five minutes to destroy the kinase. After cooling at 0° C., a 1/10 ml. aliquot is removed for ATP assay. The difference between the ATP content prior to incubation with phosphocreatine kinase and the ATP content after such incubation is the amount of ADP which has been converted to ATP.

The following examples illustrate the practice of this invention:

EXAMPLE 1

An aqueous suspension of monkey kidney tissue is divided into 10 ml. aliquots. Five of the aliquots are inoculated by adding thereto equal aliquots of monkey kidney tissue infected with an adenovirus. Both sets of cultures are incubated for the times indicated in the following table. Periodically, an aliquot is sonically ruptured and assayed for ATP as described in Procedure A, above. The results are set forth in the table.

TABLE A

Material tested	Incubation time, days	Response, light units, mv.
Virus infected, inoculated	0	45
Control, non-inoculated	0	50
Virus infected	2	125
Control	2	30
Virus infected	4	480
Control	4	10
Virus infected	19	820
Control	10	0

EXAMPLE 2

Chicken embryo tissue cultures were grown and inoculated with 1/10, 1/1000, and 1/10,000 dilutions of influenza virus, A-2, strain Jap 305. The infected and uninfected control cultures were incubated at 36° C. for 24 hours. After incubation, the fluid culture medium was removed and the tissue cell layer suspended in 1 ml. of cold 0.02 M arsenate buffer at pH 7.4. The suspension was added to a centrifuge tube and 5 ml. of cold n-butanol added. After mixing for 10 seconds the tubes were centrifuged at 500×g for 2 minutes. Aliquots 0.01 ml.) of the aqueous phase were removed and assayed for ATP using procedure A as described above. The results are shown in Table B.

TABLE B

Sample:	Response (light units), mv.
No virus	1,170
Virus, 1/10,000 dilution	1,150
Virus, 1/1000 dilution	1,120
Virus, 1/10 dilution	455

As can be seen, as the amount of virus increased, the amount of bioluminescence decreased.

EXAMPLE 3

Chicken embryo tissue cultures were inoculated with Simliki Forest Virus at dilutions of 1/100 and 1/10,000. The infected and uninfected control cultures were incubated at 36° C. for 6 hours and 24 hours. After incubation, the fluid culture medium was removed and the tissue cell layer suspended in 5.0 ml. of cold 0.02 M arsenate buffer at pH 7.4. The suspension was centrifuged at 500×g for 2 minutes and the supernatant discarded. The packed tissue cells were resuspended in cold buffer and 1.0 ml. filtered through a bacterial filter. The cells were washed with 1.0 ml. of cold buffer, treated with n-butanol and washed again with 1.0 ml. of cold buffer. The extracted solution was centrifuged at 500×g for 2 minutes and 0.01 ml. aliquot of the aqueous layer removed for ATP assay. The results of the assay are shown in Table C.

TABLE C

Sample	Incubation time, hours	Response, mv.
No virus	6	41,100
Virus, 1/10,000	6	36,000
Virus, 1/100	6	30,100
No virus	24	34,100
Virus, 1/10,000	24	30,400
Virus, 1/100	24	29,800

EXAMPLE 4

The procedure as set forth in Example 3 is followed, except Hela tissue culture cells were used and were infected with rhino virus. After 24 hours incubation, the cells were analyzed for ATP, using above-described procedure A with the following results:

TABLE D

	Response, mv.
20 HeLa cells, no virus	30,900
HeLa cells, +rhino virus	25,600

EXAMPLE 5

Rabbit kidney cells were grown and inoculated with Herpes simplex virus, overlaid with an agar medium and incubated for 24 hours. After treatment similar to that described with respect to Example 4, the extracted ATP was measured with the following results:

TABLE E

	Response, mv.
30 Rabbit kidney, no virus	71
Rabbit kidney, Herpes virus	119

The comparison of the above examples illustrate that a significant change (e.g. increase or decrease) in the amount of bioluminescence given off by a sample indicates the presence of a virus therein. No significant change, of course, indicates the absence of virus.

EXAMPLE 6

The process of Example 1 is repeated except that the material used to inoculate the tissues is a unknown suspected to contain an adenovirus. After a three day period of incubation, the bioluminescent assay indicates that the inoculated culture contains about 10 times as much ATP as the non-inoculated culture. Thus, it is established that the unknown material contains a virus.

EXAMPLE 7

Ten ml. aqueous suspensions of each of the following tissue cultures are prepared: chicken embryo, hamster kidney, monkey kidney and HeLa cells. Each of the tissue cultures is inoculated with a 1 ml. portion of water which is suspected to contain a virus. Each tissue culture is incubated for a period of several days. Before incubation and periodically during and following incubation, equal aliquots of each tissue culture are sonically ruptured and assayed for ATP as described in Procedure A, above. It is found that the ATP content of the chicken embryo tissue culture changed materially during incubation. The ATP content of all of the other tissue cultures did not change significantly. Thus, it is established that the water tested contains a virus that will reproduce in chicken embryo tissue culture but which will not reproduce in hamster kidney tissue culture, monkey kidney tissue culture or HeLa culture.

EXAMPLE 8

An aqueous suspension of chicken embryo tissue culture is divided into four 10 ml. aliquots. To each of three of the aliquots there is added a different specific antiserum for each of the following viruses: St. Louis encephalitis, yellow fever and influenza. All four aliquots are then inoculated by adding thereto 1 ml. portions of the water tested in Example 7. Each tissue culture is then

incubated for several days. Before incubation and periodically during and following incubation, equal aliquots of each of the four suspensions of tissue cultures are sonically ruptured and assayed for ATP as described in Procedure A, above. It is found that the ATP contents of the control culture (i.e., the one not containing an anti-serum) and of the cultures containing anti-serums for St. Louis encephalitis and influenza increased several fold during incubation. However, the ATP content of the culture containing anti-serum for yellow fever does not increase significantly following incubation. This indicates that the water tested contains yellow fever virus.

I claim:

1. A method for determining the presumptive presence of a virus in an unknown material which comprises inoculating a tissue culture which is an appropriate host for the suspected virus with a sample of the material suspected to contain a virus, incubating said inoculated tissue culture and measuring and comparing the ATP content of said tissue culture both before and after said incubation period, the results of said comparison indicating the presence or absence of a virus in said material.

2. The method of claim 1 wherein said ATP content is measured by mixing equal aliquots of said tissue culture, one before and one after said incubation period, with firefly lantern extract, said extract containing luciferin, luciferase and magnesium, and measuring the amount of light emitted.

3. A method for determining the presumptive presence of a virus in an unknown material which comprises providing duplicate tissue cultures which are hosts for the suspected virus, inoculating one of said tissue cultures with a sample of the unknown material suspected to contain a virus, after an incubation period mixing each of said tissue cultures in the presence of oxygen with firefly lantern extract, said extract containing luciferin, luciferase and magnesium, measuring the amount of light emitted and comparing the amount of light emitted by the inoculated tissue culture with the amount of light emitted by the non-inoculated tissue culture, the results of said comparison indicating the presence or absence of a virus in said material.

4. The method of claim 3 wherein said virus is adenovirus and wherein said host tissue is monkey kidney tissue.

5. The method of claim 3 wherein said virus is yellow fever virus and said tissue culture is chicken embryo.

6. A method for determining the presumptive presence of a virus in a host tissue which comprises providing duplicate tissue cultures which are hosts for the suspected virus, inoculating one of said tissue cultures with a sample of the host tissue suspected to contain a virus, incubating said tissue cultures, at periodic intervals treating equal portions of each of said tissue culture to rupture the cells of said tissue and extract the adenosine triphosphate therefrom and thereafter mixing said treated

cultures in the presence of oxygen with firefly lantern extract, said extract containing luciferin, luciferase and magnesium, measuring the amount of light emitted and comparing the amount of light emitted by the inoculated tissue culture with the amount of light emitted by the non-inoculated tissue culture, the results of said comparison indicating the presence or absence of a virus in said host tissue.

7. A method for determining the presumptive presence of a virus in an unknown material which comprises providing a plurality of different tissue cultures, each tissue culture being a host for a different group of viruses, inoculating each tissue culture with a sample of the unknown material suspected to contain a virus, both before and after an incubation period mixing equal aliquots of each of said tissue cultures in the presence of oxygen with firefly lantern extract, said extract containing luciferin, luciferase and magnesium, measuring the amount of light emitted and comparing the amount of light emitted by each inoculated tissue culture before and after incubation, the results of said comparison indicating the presence or absence of virus in said material and indicating which one, if any, of said tissue cultures is a host for any virus which is present.

8. The method of claim 7 wherein said plurality of tissue cultures comprise chicken embryo, hamster kidney, monkey and HeLa cells and said virus is one which causes an increase in ATP after said incubation only in said chicken embryo.

9. A method for identifying the type of virus present in a material which comprises providing a plurality of equal aliquots of a tissue culture which is a host for said virus, adding a different anti-serum to each of said aliquots, each of said anti-serums being specific for a different virus, inoculating each tissue culture with the virus, incubating each of said tissue cultures, both before and after said incubation mixing equal amounts of each of said tissue cultures in the presence of oxygen with firefly lantern extract, said extract containing luciferin, luciferase and magnesium measuring the amount of light emitted and comparing the amount of light emitted by each inoculated tissue culture before and after incubation, the results of said comparison indicating the type of virus present in said material.

10. The method of claim 9 wherein said tissue culture is chicken embryo and said virus is yellow fever virus.

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