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[54] **TEST FOR SYSTEMIC LUPUS ERYTHEMATOSUS**

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[56] **References Cited**

UNITED STATES PATENTS

3,555,143 1/1971 Axen et al. ..... 23/230 B

OTHER PUBLICATIONS

Wold et al., Science, v. 161, pp. 806–807, (1968).

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[57] **ABSTRACT**

A test for systemic lupus erythematosus which involves the detection of anti-DNA antibodies by use of ^{125}I labeled DNA. According to the test, native DNA labeled with ^{125}I labeled 5-iododeoxyuridine (IUdR) preferably, is mixed with a sample of serum from a patient to be tested. The mixture is then incubated to allow reaction or complexing of the DNA with any anti-DNA antibodies present in the serum, after which a precipitating agent for antibody/DNA complexes is added and the supernatant and precipitate fractions are separated. The percentage of DNA bound to antibodies may then be readily determined by calculation from the respective radioactivities of the supernatant and precipitate fractions. A high percentage of bound DNA represents a positive test for systemic lupus erythematosus.

9 Claims, No Drawings

TEST FOR SYSTEMIC LUPUS ERYTHEMATOSUS

BACKGROUND OF THE INVENTION

The present invention relates to a test for systemic lupus erythematosus. More particularly, the invention is directed to methods for detecting anti-DNA antibodies in blood serum and the like.

Systemic lupus erythematosus (SLE) is a serious autoimmune disease which affects the collagen of the connective tissues of the human body. Since SLE may affect one or more of a large number of different tissues or organs of the body, such as blood vessels, heart, kidneys, skin, serous membranes, etc., clinical diagnosis of SLE is often difficult since the symptoms may resemble a number of other diseases such as rheumatoid arthritis, skin cancer, serum sickness, rheumatic fever, multiple myeloma, Sjogren's syndrome, etc.

It is important to be able to distinguish SLE from other diseases due to the high probability of kidney damage from the disease. Thus, lupus nephritis is very frequent in the disease and is the most serious feature of SLE. Such nephritis is a frequent cause of death in SLE patients, and consequently it would be most advantageous to be able to detect SLE early enough to prevent or mitigate serious kidney damage.

It has been demonstrated that serums from patients with connective tissue disorders contain antibodies to many nuclear constituents. In particular, circulating antibodies against native DNA (deoxyribonucleic acid) have been found to be highly specific to patients suffering from systemic lupus erythematosus. The appearance and exacerbation of the nephritis attendant in SLE appears to be related to the formation and deposition of antibody/DNA complexes in the kidneys and particularly the renal glomeruli. Although the exact reasons for the production of these antibodies and the reasons for their particular specificity to SLE are as yet unknown, the detection and measurement of these anti-DNA antibodies has become increasingly important in the clinical diagnosis and evaluation of this disease.

Recently, a precise method for measuring various small amounts of anti-DNA antibody has been developed using ^{14}C labeled DNA. See T. Pincus et al., "Measurement Of Serum DNA-Binding Activity In Systemic Lupus Erythematosus," 281 New England Journal of Medicine, 701–705 (1969). This method appears to be the most definitive test for SLE presently available. It is reported to be positive in 75 percent of SLE patients studied, whereas other leading tests were positive in only about 25–64 percent of SLE cases. Moreover, this anti-DNA antibody test has shown greater specificity for SLE than other diagnostic tests used, such as immunodiffusion, complement-fixation, precipitation, etc.

The test for SLE using ^{14}C labeled DNA is based upon the differential solubility of free DNA and DNA-immunoglobulin (antibody) complexes in ammonium sulphate solution. Whereas DNA is soluble in a 50 percent saturated ammonium sulphate solution, immunoglobulins or antibodies and immunoglobulin-bound DNA (hereinafter referred to as antibody/DNA complexes or anti-DNA/DNA complexes) are insoluble in this solution. If a mixture of ^{14}C labeled DNA and serum containing DNA antibody is rendered 50 percent saturated with ammonium sulphate, radioactive label appears in the precipitate on binding of DNA to the immunoglobulins. The percentage of radioactive

label appearing in the precipitate provides a measure of the antibody present (antibody bound to DNA). For example, after centrifuging to separate the precipitate and supernatant liquid, half of the supernatant liquid may be taken off as the supernatant fraction with the remaining supernatant liquid and the precipitate constituting the precipitate fraction. The radioactivity of the respective fractions can then be determined in a liquid scintillation counter and the DNA binding activity can be calculated by the following formula:

$$100 \times \frac{P-S}{P+S} = \%\ \text{DNA bound}$$

Where P is the radioactivity in the precipitate fraction and S is the radioactivity of the supernatant fraction.

Although the ^{14}C labeling of DNA provides a precise and relatively simple method for testing for systemic lupus erythematosus, there are a number of serious disadvantages to this test. First of all, the preparation of ^{14}C labeled DNA is difficult to perform. Secondly, the preparation of samples for ^{14}C - counting is very laborious. Thirdly, and more importantly, this method cannot be performed in most nuclear medicine laboratories because of the expensive and complex radioactive detection equipment needed with ^{14}C labeling. Thus, ^{14}C emits a low energy beta particle only, and therefore, very complex beta particle counting mechanisms, such as liquid scintillation counters, must be used. Such complex and expensive equipment is not available in most nuclear medicine laboratories and the usual gamma counting equipment present in most nuclear medicine laboratories cannot be used for ^{14}C measurements.

Accordingly, it is evident that it would be highly advantageous to develop a method for testing for SLE which could be performed by most laboratories using conventional gamma counting equipment. It would also be advantageous to have a method involving a simpler procedure for making radioactive labeled DNA.

BRIEF SUMMARY OF THE INVENTION

The above and other disadvantages of the prior art are overcome or alleviated by the improved method of the present invention which comprises labeling a sample of native DNA with ^{125}I, mixing the ^{125}I-labeled DNA with a sample of serum from a patient to be tested, incubating the resulting mixture to allow reaction or complexing of the ^{125}I-labeled DNA and any anti-DNA antibodies present in the serum, precipitating the antibody/DNA complexes and separating the supernatant and precipitate fractions to determine the respective radioactivities of the two fractions, and calculating the percentage of DNA bonding with antibodies on the basis of the relative radioactivities of the fractions, a high percentage of DNA bonding representing a positive test for systemic lupus erhthematosus.

The method of the present invention includes two different methods for preparing the ^{125}I-labeled DNA reagent. In the preferred method of the present invention, native (double stranded) DNA is labeled with ^{125}I by growing a source of DNA, such as E. coli cells, in the presence of ^{125}I-labeled 5-iododeoxyuridine (IUdR), in order to incorporate ^{125}I-labeled IUdR into the growing DNA as will be discussed more fully below. Although this preferred method is somewhat more laborious than the alternate method referred to below in terms of

eparation of reagent, it yields a superior product and s been found to be at least as sensitive and specific the method involving ^{14}C labeling of DNA.

The alternate method of ^{125}I labeling used in the ethod of the present invention comprises direct iemical iodination of native DNA with ^{125}I-. As will be scussed more fully below, direct chemical iodination ay be carried out with a sodium salt of ^{125}I in the pres- ice of an aqueous solution of potassium iodide and allium thrichloride oxidizing agent, buffered to a pH about 5. Although the preparation of ^{125}I-labeled NA by direct chemical iodination is much simpler an the preferred method indicated above, the result- g reagent provides a less sensitive or less specific test r SLE, because of inevitable labeling of trace protein ipurities from the DNA preparation.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The labeling of biological materials, including DNA ith radioactive iodine (^{125}I) has previously been re- orted in the literature. See for example S. L. Commer- rd, "Iodination Of Neuclic Acids In Vitro," Biochem- ry, Volume 10, No. 11, pages 1993–2,000 (1971) id J. Wikman-Coffelt, "Immunochemical Analyses Of ellular DNA," Biochemical And Biophysical Re- arch Communications, Volume 48, No. 3, pages 01–507 (1972). However, such radioactive labeling of NA with ^{125}I has to our knowledge only been used in NA research, particularly DNA analysis and synthe- s, etc. The possibilities and advantages of using ^{125}I- beled DNA in testing for systemic lupus erythema- sus have not to our knowledge been previously recog- zed in the art.

Radioactive iodine (^{125}I) has a half life of 60 days, id as a result can be used to prepare reagents having shelf life of about 3 or 4 months (approximately two alf lives). ^{125}I is commercially available in several rms including sodium iodide (Na ^{125}I), available om New England Nuclear Corporation, and -iododeoxyuridine (^{125}I-UdR), available from mersham/Searle.

ed out by incorporating the $^{125}IUdR$ into DNA in owing cells. For example, E. coli cells, strain 15 TAU ay be used as the source of DNA. The $^{125}IUdR$ may e incorporated into the E. coil cells generally analo- ous to the method employed by Hanawalt for prepar- g 5-bromouracil labeled DNA (see P. C. Hanawalt, Preparation of 5-Bromouracil-labelled DNA," in rossman, Methods In Enzymology, New York, Aca- emic Press (1967), Volume 12, Part A, pp. 702–708). detailed example of carrying out this procedure in onnection with the present invention is set forth in Ex- mple I. Although $^{125}IUdR$ labeling is most efficient in thymine-requiring mutant, such as the 15 TAU strain f E. coli, it is not intended to limit the present inven- on to this source of DNA, and other microorganisms hich would incorporate IUdR efficiently may be suit- ble. The 15 TAU strain of E. coli is preferred since the ild type of E. coli will not incorporate sufficient radio- ctivity in its DNA.

In general, the preparation of $^{125}IUdR$-labeled DNA ccording to the above mentioned method is carried ut by growing E. coli cells, strain 15 TAU, on a mini- al medium such as that of Davis et al. (see B. D. Davis t al., "Mutants of E. Coli Requiring Methionine Or Vi- amin B_{12}, " 60 Journal of Bacteriology 17–28 (1950).

The minimal medium of Davis comprises a solution of the following components in one liter of water:

7g potassium monophosphate ($K_2HPO_4.3H_2O$)
3g potassium diphosphate ($K\ H_2\ PO_4$)
0.5g sodium citrate ($Na_3\ C_6\ H_5\ O_7.2H_2O$)
0.1g magnesium sulphate ($Mg\ SO_4.\ 7H_2O$)
1g ammonium sulphate ($(NH_4)_2\ SO_4$)

If desired the above solution may be concentrated 20 times by making up the solution in 50 mls of water and diluted as necessary. As in the method of Hanawalt the medium is supplemented with the following:

40% glucose solution
2.5 mg/ml thymine (5-methyluracil)
2.0 mg/ml DL-arginine
2.0 mg/ml uracil

The above supplements are added to the medium at the rate of 1 ml each per 100 mls of medium.

The E. coli cells are grown in a culture containing the Davis medium plus supplements, and growth of the cells is measured by a colorimeter, such as the Spectronic 20 made by Bausch and Lomb, Inc. When the culture of cells reaches an O.D. (optical density) at 450 μ of 0.70–0.75, the cells are harvested, washed and transferred to fresh medium containing the same supplement except that only 0.1 ml of 1 mg/ml thymine is added to 100 ml of medium (i.e., thymine concentration of 1 μg/ml), and 0.4 percent glucose is present in the medium. To this fresh medium is then added 200–500 microcuries (μCi) of ^{125}I-labeled 5-iododeoxyuridine (approximately 20–25 μg $^{125}IUdR$). The culture is then grown to an O.D. of 0.7–0.8, and the cells are harvested, washed and suspended in the NET buffer of Hanawalt (0.1M NaCl; 0.01M EDTA; 0.01M Tris-Cl, pH8.0).

The cells are then lyzed with 1 percent sodium dodecyl sarcosinate (SDS) by heating at 60°C. until the solution clears (about 5–7 minutes). The DNA is then extracted and purified such as by the method of Marmur (see J. Marmur, "A Procedure For The Isolation Of Deoxyribonucleic Acid from Micro-organisms," 3 Journal of Molecular Biology 208–218 (1961)). This method comprises repeated extractions with a chloroform-octanol system. Partially purified DNA may be obtained by isopropanol precipitation following the first chloroform extraction. It has been found that extensive purification is not essential to obtain good sensitivity and specificity in the test for systemic lupus erythematosus. Thus, although DNA which is only partially purified will still contain protein and RNA, these impurities do not affect the antibody test since they are not labeled with $^{125}IUdR$ by the above procedure. If, however, it is desired to obtain extensively purified DNA, the material is extracted until no protein is visible at the interface of the chloroformoctanol system, and the extracted DNA is then treated with RNAse and pronase, such as according to the system of Pincus et al., supra.

After the final isopropanol precipitation to obtain partially purified DNA, the yield from a 100 ml culture as prepared above is about 100 μg DNA with a specific activity of about 30,000–50,000 counts per minute per microgram (cpm/μg). As indicated in the specific examples below, it is important that the radioactivity of the labeled DNA reagents used in the method of the present invention be kept to a minimum (i.e., about 10,000–50,000 cpm/μg) in order to avoid excessive internal irradiation. Thus, it has been found that much

higher specific radioactivities and prolonged storage result in increased binding of the labeled DNA especially in normal serum. Although applicants do not wish to be bound by any particular theory, since internal irradiation is responsible for reduced molecular weight of the DNA, which in turn may cross link with protein impurities, it is believed that more of these proteins may precipitate out in the ammonium sulphate solution.

An alternate method of preparing ^{125}I-labeled DNA reagent for use in the method of the present invention comprises the direct chemical iodination of DNA. The chemical iodination may be carried out generally analogous to the method of Commerford, supra. Although a number of different sources of DNA may be used for labeling and use in the method of the present invention, highly polymerized salmon sperm DNA is most preferred because it contains the least amount of protein traces. Salmon sperm DNA is commercially available from Mann Research Laboratories.

The highly polymerized salmon sperm DNA is dissolved in a 1/10th (dilution) SSC buffer solution (0.15M NaCl and 0.015M $Na_3C_6H_5O_7.2H_2O$, pH 7.1) to the extent of 0.4 percent by weight. 10 microliters of the DNA solution are then diluted with 200 microliters (μl) of ammonium acetate buffer (0.1M ammonium acetate adjusted to pH 5.0 with glacial acetic acid). Next there are added 25 μl of 2.5×10^{-4}M potassium iodide (KI), 50 μCi of carrier-free radioactive sodium iodide ($Na^{125}I$) and 25 μl of 1.5×10^{-3}M thallium trichloride ($TlCl_3.H_2O$). The thallium trichloride serves as an oxiding agent, and is commercially available from K & K Laboratories.

The above mixture is then incubated at 60°C. for 15 minutes, followed by chilling in ice. The excess thallium trichloride is then neutralized with 25 μl of 0.01M sodium sulfite (Na_2SO_3). This neutralized reaction mixture is then dialyzed three times against 1 liter portions of cold ammonium acetate buffer (pH of 5), followed by dialysis two times with borate buffer (0.16M Na Cl and 0.2M H_3BO_3, adjusted to pH 8.0 with solid Na OH).

Under the above conditions, the specific radioactivity of the ^{125}I-labeled DNA recovered is 8,000–10,000 cpm/μg. The only iodinated residue is cytosine. As with the ^{125}IUdR labeling of DNA, the direct iodination of DNA should be carried out only to such a degree as will yield a specific radioactivity low enough to avoid as much as possible the adverse effects of internal irradiation upon prolonged storage. Whereas a specific radioactivity range of about 10,000–50,000 cpm/μg, and preferably 30,000–50,000, is best for ^{125}IUdR-labeled DNA, chemically iodinated ^{125}I-labeled DNA should have a specific radioactivity range of about 8,000–10,000 cpm/μg. Higher specific activities yield a heavily irradiated product, while lower specific activities, although usable, are inconvenient (longer counting times and less accuracy).

Moreover, as indicated more clearly in the specific examples below, it has been found preferable to limit the extent of the direct iodination of DNA by utilizing a minimum amount of iodine. This may be done by using a potassium iodide concentration in the above reaction mixture of between about 1×10^{-5}M and 5×10^{-5}M, and preferably about 2.5×10^{-5}M potassium iodide. Aside from the two above parameters, it will be understood that the above procedure may be varied within reasonable limits, and substituting various reagents, as will be obvious to those of ordinary skill in the art.

According to the method of the present invention, the above described ^{125}I-labeled DNA reagents are used to test samples of blood serum for SLE in a manner analogous to that of Pincus et al., supra. The method of the present invention comprises mixing the blood serum to be tested with one of the ^{125}I-labeled DNA reagents in a buffer solution, adjusted to a pH of about 8.0. This may suitably be carried out by preparing a solution of 0.1 to 0.5 μg ^{125}I-labeled DNA in a 0.15M borate-sodium chloride buffer (pH 8.0), and mixing 50 microliters of this solution with an equal amount of serum solution prepared by diluting 5 microliters of serum with the same buffer. This mixture is thereafter incubated at 37°C. for 30 minutes and then stored in the refrigerator overnight. This incubation and storage period allows sufficient time for the DNA to complex with any anti-DNA antibodies present in the serum.

Next, a suitable precipitating agent is added to the mixture which will precipitate antibody/DNA complexes but which will not precipitate non-complexed DNA. As indicated by Pincus et al., a 50 percent saturated solution of ammonium sulphate will precipitate immunoglobulins and immunoglobulin-bound DNA, whereas unbound DNA is soluble in this solution. A suitable method of carrying out this precipitation is to add to the mixture of serum and ^{125}I-labeled DNA an equal volume (i.e., 100 μl) of cold saturated ammonium sulphate. After thorough mixing and standing in the cold for one hour, the mixture may be centrifuged to separate the mixture into supernatant liquid and precipitate. After centrifugation, 100 ul (i.e., approximately half) of the supernatant liquid is removed as the supernatant fraction and the radioactivity is counted in a suitable gamma type counting device such as a Baird Atomic Spectrometer. Similarly, the radioactivity of the remaining supernatant liquid containing the precipitate (which together constitute the precipitate fraction) is also counted in the same gamma counting device, and the binding or comlexing of DNA is calculated according to the following formula:

$$P-S/P+S \times 100 = \text{Percent DNA bound}$$

Wherein s is the radioactive count of the removed supernatant fraction and P is the radioactive count of the precipitate.

Since, as previously explained, anti-DNA antibodies circulating in the blood are now considered to be a fairly accurate indicator of SLE disease, a high degree of radioactivity in the precipitate fraction (which translates into a high percentage of bound DNA according to the above formula) is considered to represent a positive test for systemic lupus erythematosus according to the method of the present invention. Exactly what constitutes a high degree of radioactivity in the precipitate fraction varies depending upon which of the two reagents described above is used in the method of the present invention. According to the preferred method, where ^{125}IUdR-labeled DNA is used as the reagent, the test is very sensitive, and a normal serum will generally give a percent DNA bound of very close to zero. Accordingly, using this preferred reagent, a percent DNA bound of anything greater than about 20 percent is considered to be a positive test for SLE.

On the other hand, where the DNA is labeled with ^{125}I by direct chemical iodination, the test is much less sensitive, and a normal serum may show a percent DNA bound of as much as 30 percent and the SLE serum may give relatively low binding values, as low as about 40 percent. Accordingly, where this reagent is used a percent DNA bound of up to about 30 is considered a negative test for SLE and anything greater than 40 percent is considered a positive test. These results are due to the presence of small amounts of protein impurities (in the DNA) which get the ^{125}I label since proteins are better substrates for iodination than nucleic acids. These proteins precipitate with ammonium sulphate (see Example III below) and alter the (P—S)/(P+S) ratio. Although this alternate method in which DNA is labeled by direct chemical iodination is less sensitive than the preferred method, the preparation of reagent is simpler and can be readily carried out by most laboratories.

The method of testing for SLE according to the present invention has been found to be at least as accurate as the method of Pincus et al. using ^{14}C labeled DNA. Thus, in over 100 determinations on SLE positive serums (determined by ^{14}C labeled DNA), the test method of the present invention has yielded no false negatives. As with the ^{14}C method, false negatives are encountered with the test method of the present invention were an SLE patient is under treatment so that the disease is not in an active state but is in remission. With respect to false positives, these are quite difficult to determine since a patient yielding a false positive may have a related disease such as rheumatoid arthritis or others of the collagen diseases mentioned previously. Since the exact progression of SLE is still unknown, it is suspected that some or all of these false positive patients may in fact be developing SLE.

Of almost equal importance to the accuracy of the test method of the present invention is the fact that it requires less complicated equipment and less complicated procedures for making the reagent. As a result, the test can be carried out by most laboratories with conventional gamma counting equipment.

The method of the present invention will now be described in further detail with reference to the following specific, non-limiting examples:

EXAMPLE I

Four different samples of ^{125}I labeled DNA were prepared according to the following methods:

1. E. coli cells, strain 15 TAU were grown as described previously (analogous to the Hanawalt method for preparation of 5-bromouracil labeled DNA) on a Davis minimal medium supplemented with 25 μg/ml thymine, 50 μg/ml arginine and 20 μg/ml uracil. When the culture reached O.D. 450 μ of 0.7, the cells were harvested, washed and transferred to fresh medium containing only 1 μg/ml thymine and 200 μCi of ^{125}I-UdR (approximately 20 μg). The culture was then grown to an O.D. of 0.7–0.8 (a duplicate bottle was prepared without the $^{125}IUdR$ and optical density readings were taken on this non-radioactive bottle). Readings were taken often in order to make sure that the culture did not go beyond 0.8 O.D., and it took approximately one-and-a-half hours to reach this final turbidity. The cells were then harvested by centrifuging at about 5,000 rpm for ten minutes in the cold and then washed by re-suspending in 30 mls of ice cold NET buffer and again centrifuged. The washing and centrifuging was repeated two more times and the final washed pellet had a count of about 400,000 counts per 10 seconds on top of well counter. The cells were then resuspended in 2 mls of NET buffer, transferred to extraction tubes and frozen overnight. After thawing the suspension, 0.2 mls of SDS was added and the suspension was heated at 60°C. for three to ten minutes. The clear solution was then cooled to room temperature and 0.5 mls of 5M Na Cl O_4 were added. The DNA was then extracted by adding 2.5 mls of chloroform-octanol (9:1), shaking, placing on a rotating plate for 20 minutes, centrifuging in a Servall (swinging bucket) at 5,000–6,000 rpm for ten minutes, and finally separating the top aqueous layer from the bottom organic layer. Partially purified DNA was then obtained by isopropanol precipitation from aqueous layer.

2. An extensively purified sample of $^{125}IUdR$ labeled DNA was obtained by the same method as in paragraph (1) above, except that the extraction procedure with chloroform-octanol was repeated until no protein was visible at the interface. The extracted DNA was then treated with RNAse and pronase according to the method of Pincus et al. In the highly purified samples, the final yield from a 100 ml culture to which the ^{125}I-UdR was added was approximately 96 μg DNA with a specific activity of about 9,400 cpm/μg.

3. A sample of chemically iodinated ^{125}I-labeled DNA was prepared by adding 0.2 mls of ammonium acetate buffer (pH 5.0) to 10 μls of a solution containing 40 μg of salmon sperm DNA obtained from Mann Research Laboratories. To this solution were added 25 μl of 2.5×10^{-4}M KI containing 50 μCi of carrier-free $Na^{125}I$, and 25 μl. of 1.5×10^{3}M $TlCl_3$. This mixture was incubated for 15 minutes at 60°C., and then chilled in ice. After neutralizing excess $TlCl_3$ with 25 μl of 0.01M Na_2SO_3, the reaction mixture was dialyzed three times against 1 liter portions of cold ammonium acetate buffer, followed by 2 runs with borate buffer (pH 8.0). The DNA recovered had a specific activity of between 8,000 and 10,000 cpm/μg.

4. Another ^{125}I-labeled sample of DNA was prepared with DNA extracted from cold E. coli cells, but labeled by direct chemical iodination instead of $^{125}IUdR$. The procedure used was the same as that described in paragraph (3) above.

Each of the above reagents was then used to test samples of blood serum from both normal and SLE patients, the tests being run according to the method of the present invention as described previously. The results of these tests are summarized in Table I below. As can be seen from Table I, DNA labeled with $^{125}IUdR$ incorporated into E. coli cells according to the preferred method of the present invention showed excellent sensitivity for SLE serum. Thus, whether or not the $^{125}IUdR$ labeled DNA was purified, the test of the present invention showed no binding in normal serum and almost 100 percent binding in SLE serum. The test run with chemically iodinated ^{125}I-labeled DNA from salmon sperm was not as sensitive, but still showed a relatively clear specificity for SLE serum. The ^{125}I-labeled DNA by chemical iodination from E. coli cells gave very high binding in both normal serum and SLE serum, since it contained a significant amount of contaminating proteins.

TABLE I

No.	Labelling	DNA Source	Protein Present	% Binding Normal	% Binding SLE
(1)	^{125}IUdR	E. coli, 15 TAU	~20% (not purified)	0	93
(2)	^{125}IUdR	E. coli, 15 TAU	0.5% (purified)	0	98
(3)	^{125}I (chemical) iodination)	Salmon sperm	—	25	74
(4)	^{125}I (chemical) iodination)	E. coli, 15 TAU	1.0%	65	62

EXAMPLE II

Partially purified ^{125}IUdR-labeled DNA was prepared according to the same method as described in paragraph (1) of Example I. This reagent was used to test 31 samples of blood serum (23 normal and 8 SLE). For each sample of blood serum, the test was repeated 4 times and the average of the four determinations was used as the final percent binding value for that sample. For comparison, ^{14}C labeled DNA was prepared from KB cells according to the method described in Pincus et al., supra. The same 31 blood serum samples were then tested with this ^{14}C labeled DNA according to the method of Pincus et al., as described previously. Again, the average of four determinations was used. Table II summarizes the results of these tests giving both the average and the range of the values for each of the 23 normal sera and each of the 8 SLE sera. The results indicate that the ^{14}C determination of the prior art and the test method of the present invention are comparable in both sensitivity and specificity.

TABLE II

Reagent-Test	Normal % Binding 23 sera Avg.	Normal % Binding 23 sera Range	SLE % Binding 8 sera Avg.	SLE % Binding 8 sera Range
^{14}C-DNA Pincus et al.	0	−6 to +7	70	60 to 76
^{125}IUdR-DNA present invention	1	−1 to +3	97	92 to 98

EXAMPLE III

A further experiment was performed to study the effect of the presence of labeled protein by adding trace amounts of ^{125}I-labeled human gamma-globulin and human albumin, B grade to ^{125}IUdR-labeled DNA. The human albumin (B grade) and human gammaglobulin (fraction II) were obtained from Calbiochem and were labeled with ^{125}I by direct chemical iodination according to the procedure described in paragraph (3) of Example I. The ^{125}IUdR-labeled DNA was prepared by the same method as in paragraph (2) of Example I. The results of the tests on both normal and SLE sera using the method of the present invention are summarized in Table III. This table shows that the addition of labeled gamma globulin raises the percent binding in normal serum and lowers the percent binding in SLE serum, while albumin, which does not precipitate with ammonium sulphate, has no effect. This is consistent with the observations on directly iodinated DNA (see Sample (3) of Example I). That is, the apparent cause of both high normal values and low SLE values is the presence of protein impurities in the DNA. Since protein is a much better substrate for iodination than DNA, even a small amount of protein is labeled disproportionately with ^{125}I. Because of the difficulty in removing all protein by purification procedures, ^{125}IUdR labeling of DNA is preferred.

TABLE III

Reagent	% Binding Normal	% Binding SLE
^{125}IUdR-DNA (2000 cpm)	0	75
^{125}IUdR-DNA (2000 cpm) + ^{125}I-gamma globulin (700 cpm)	13	48
^{125}IUdR-DNA (2000 cpm) + ^{125}I-albumin (900 cpm)	1	68

EXAMPLE IV

In order to demonstrate the effect of varying the potassium iodide concentration during the direct chemical iodination of DNA, a number of runs were made with different concentrations of KI. From the results set forth in Table IV below, it can be seen that although binding of ^{125}I labeled DNA in normal serum is not affected, the binding in SLE serum appears to decrease with increasing concentrations of KI (i.e., increasing iodination of DNA). Accordingly, when using direct chemical iodination, the potassium iodide concentration should be kept at about 1×10^{-5}M to 5×10^{-5}M and preferably about 2.5×10^{-5}M.

TABLE IV

KI concentration	Specific activity (cpm/μg)	% Binding Normal	% Binding SLE
2.5×10^{-5}M	3510	15	59
2.5×10^{-4}M	8600	31	42
2.5×10^{-3}M	4330	26	23

EXAMPLE V

The effect of self-irradiation or internal irradiation of ^{125}I-labeled DNA prepared by chemical iodination was measured by both molecular weight determinations and by the effect on DNA binding in normal and SLE sera. The molecular weight determinations were performed by analytical ultra-centrifugation and the band sedimentation method of Studier (see F. W. Studier, "Sedimentation Studies of the Size and Shape of DNA," 11 Journal of Molecular Biology, 373–390 (1965)). Thus, in each case 10 μl of solution containing approximately 1 μg of DNA was placed in a sample well with the bulk solution comprising 1M NaCl, 0.01M Tris-Cl (pH 7.6). Each run was carried out at 25,980 rpm and 20°C. with an initial slow acceleration at 5,000 rpm for 3 to 5 minutes. The films were scanned with a Gilford Spectrophotometer, Model 2400, equipped with a linear transport attachment. The position of the band was measured in the scans and the

olecular weights were calculated according to udier. Molecular weight determinations and tests acrding to the present invention were made on 5 sames as shown in Table V below. The number of days dicated is the storage time for the ^{125}I-labeled DNA. ıe results summarized in the Table indicate that self-adiation produced no substantial effect on SLE bindg but showed a marked increase in normal serum nding. Since the molecular weight of DNA was reıced by self-irradiation, the increased binding in norıl serum may be due to non-specific binding to ıaller DNA fragments.

TABLE V

ecific Activity (cpm/μg)	M.W. of DNA ($\times 10^{-6}$)	% Binding Normal	% Binding SLE
No iodination	6.54	—	—
3545 (1 day)	3.55	7	66
3545 (10 days)	2.85	22	81
236,000 (1 day)	1.74	29	76
236,000 (10 days)	0.81	46	87

EXAMPLE VI

Tests were also run to determine the reactivity of the tibodies with native DNA compared to denatured NA (labeled prior to denaturation). Table VI shows e percent binding of normal and SLE sera according the method of the present invention using chemically dinated (^{125}I), native DNA and heat (about 97°C.) natured DNA. From the Table, it can be seen that natured DNA is somewhat less reactive than native NA.

TABLE VI

^{125}I-DNA (chemical iodination)	% Binding Normal	% Binding SLE
Native	33	74
Denatured	14	40

The present invention may be embodied in other speic forms without departing from the spirit or essenıl attributes thereof, and, accordingly, reference ould be made to the appended claims, rather than to e foregoing specification as indicating the scope of e invention.

We claim:

1. A method for testing patients for systemic lupus ythematosus comprising the steps of taking a sample serum from a patient to be tested, labeling a sample of native DNA with ^{125}I by introducing ^{125}I-labeled 5-iododeoxyuridine into the DNA molecule, mixing together, in the presence of a buffer, portions of said ^{125}I-labeled DNA and said serum, incubating the resulting mixture to allow complexing of the DNA with any anti-DNA antibodies present in the serum, adding to said mixture a precipitating agent for antibody/DNA complexes which precipitating agent will not precipitate non-complexed DNA, separating the mixture into supernatant and precipitate fractions, and determining the respective radioactivities of the separated fractions, a high degree of radioactivity in the precipitate fraction indicating substantial complexing of DNA with antibodies which represents a positive test for systemic lupus erythematosus.

2. A method according to claim 1 wherein the source of DNA to be labeled is growing E. coli cells.

3. A method according to claim 2 wherein the growing E. coli cells comprise the 15 TAU strain of E. coli.

4. A method according to claim 1 wherein the source of DNA to be labeled is a thymine-requiring mutant.

5. A method according to claim 1 wherein only enough ^{125}I-labeled 5-iododeoxyuridine is introduced to yield a final specific radioactivity of the labeled DNA of about 10,000–50,000 cpm/μg.

6. A method according to claim 1 wherein a percentage of DNA binding to antibodies of greater than 20 percent represents a positive test for systemic lupus erythematosus.

7. A method for testing serum for anti-DNA antibodies comprising the steps of labeling a sample of native DNA with ^{125}I by introducing ^{125}I-labeled 5-iododeoxyuridine into the DNA molecule, mixing said ^{125}I-labeled DNA with a sample of serum to be tested, incubating the resulting mixture to allow reaction of the DNA with any anti-DNA antibodies present in the serum, adding to said mixture a precipitating agent for antibody/DNA complexes which precipitating agent will not precipitate noncomplexed DNA, separating the mixture into supernatant and precipitate fractions, and determining the respective radioactivities of the separated fractions, whereby the percentage of DNA bound to antibodies can be calculated on the basis of the relative radioactivities of the separated fractions.

8. A method according to claim 7 wherein the source of DNA to be labeled is a thymine-requiring mutant.

9. A method according to claim 8 wherein the source of DNA to be labeled comprises the 15 TAU strain of E. coli.

* * * * *

UNITED STATES PATENT OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 3,897,212

DATED : July 29, 1975

INVENTOR(S) : Shalom A. Leon, Bernard Shapiro and George J. Kollmann

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 3, line 43 before "ried out" insert the following --The preparation of ^{125}IUdR-labelled DNA may be car- --.

Column 4, line 23 delete "μ" and substitute therefor --mμ--.

Column 7, line 55 delete "μ" and substitute therefor --mμ--.

Signed and Sealed this

second Day of March 1976

[SEAL]

Attest:

RUTH C. MASON
Attesting Officer

C. MARSHALL DANN
Commissioner of Patents and Trademarks