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(54) **Title:** METHODS FOR DETECTION OF CHLAMYDIA IN SAMPLES

(57) **Abstract:** Abstract Methods for Detection of Chlamydia in Samples This invention relates to assays for Chlamydia which include the step of inactivating lipid oxidation activity in a biological sample, such as a blood or serum sample. This inactivation improves the detection of Chlamydia antigens or anti-Chlamydia antibodies. Methods and materials for the detection of Chlamydia and Chlamydial infection are provided.

Methods for Detection of Chlamydia in Samples

This invention relates to the detection of Chlamydia in samples of blood or serum. This may be useful, for example, in testing an individual for Chlamydia infection.

Chlamydiae are obligate intracellular microorganisms which parasitize eukaryotic cells and are ubiquitous throughout the animal kingdom. Chlamydiae have a biphasic developmental cycle, which has intracellular and extracellular stages, each stage having distinct morphological forms. In intracellular stages, Chlamydiae may form a metabolically-active, replicating organism known as the reticulate body (RB) or a persistent, non-replicating organism known as the cryptic phase. In infectious extracellular stages, Chlamydiae may form a metabolically-inactive body known as the elementary body (EB).

EBs are small (300-400 nm), infectious, spore-like forms which are metabolically inactive and non-replicating. They are usually found in acellular environments. EBs are resistant to a variety of physical insults, including enzyme degradation, sonication and osmotic pressure. Under oxidizing conditions in acellular environments within the host, the outer membrane of EBs is relatively impermeable and resistant to inactivation. EBs are thus well suited to survive long enough outside of their hosts to be transmitted to a new host in the form of a droplet nuclei (Theunissen et al., Applied Environmental Microbiology, 59:2589-2593 (1993)) or a fomite (Fasley et al., The Journal of Infectious Diseases, 168:493-496 (1993))

Infection by members of the genus Chlamydiae induces a significant inflammatory response at the cellular level. Clinically, the initial infection is frequently varied in symptomatology and may even be asymptomatic. Once fully

established, *Chlamydiae* are difficult to eradicate, with frequent relapse following antibiotic therapy. Evidence also indicates that the *Chlamydia* may become dormant and are then shed in quantities too few to reliably detect by culture. Chlamydial infections are therefore often chronic and persistent and there is a need for reliable, accurate methods for the diagnosis of infection.

The present inventor has recognised that inactivating or abrogating lipid oxidation activity in a biological sample, such as a blood or serum sample, increases the reliability of assays for Chlamydia in the sample.

One aspect of the invention provides a method of measuring the level of a first Chlamydia binding pair member in a sample comprising;

abrogating lipid oxidation activity in the sample, and

determining the binding of a first Chlamydia binding pair member in the treated sample to a second Chlamydia binding pair member,

the amount of binding after said treatment being indicative of the level of the first Chlamydia binding pair member in the sample.

The first Chlamydia binding pair member may be one of a Chlamydia antigen and an anti-Chlamydia antibody and the second Chlamydia binding pair member may be the other of a Chlamydia antigen and an anti-Chlamydia antibody.

For example, the first Chlamydia binding pair member may be an anti-Chlamydia antibody and the second Chlamydia binding pair member may be a Chlamydia antigen.

A method of measuring anti-Chlamydia antibody levels in a sample may comprise;

abrogating lipid oxidation activity in said sample, and;

determining the binding of antibodies in the treated sample to a Chlamydia antigen,

the amount of binding after said treatment being indicative of the level of anti-Chlamydia antibodies in the sample.

In other embodiments, the first Chlamydia binding pair member may be a Chlamydia antigen and the second Chlamydia binding pair member may be an anti-Chlamydia antibody.

A method of measuring Chlamydia antigen levels in a sample may comprise;

abrogating lipid oxidation activity in said sample, and

determining the binding of Chlamydia antigen in the treated sample to an anti-Chlamydia antibody,

the amount of binding after said treatment being indicative of the level of Chlamydia antigen in the sample.

The presence or amount of Chlamydia antigen or anti-Chlamydia antibody in a sample obtained from an individual may be indicative of the presence of Chlamydia infection in the individual.

A sample may comprise plasma or serum from the individual, and may be, for example, a blood, serum or plasma sample. Methods for obtaining, storing and preparing suitable samples from an individual are well known in the medical practice. For example, a test sample of serum may be obtained by extracting blood from an individual and isolating the serum from the extracted blood. Suitable extraction methods include centrifugation to separate serum and plasma from cellular material.

A Chlamydia antigen may be any immunogen or immunogenic component of a Chlamydia cell i.e. a molecule from Chlamydia which evokes or is capable of evoking an immune response in a mammal against the Chlamydia cell. In other words, the Chlamydia antigen is a component of a Chlamydia cell that is capable of specifically binding to antibodies raised against the Chlamydia cell. In some embodiments, the Chlamydia antigen may be LPS.

In some embodiments, the Chlamydia antigen may be an antigen on the surface of a Chlamydia cell. In other words, the binding of antibodies to a Chlamydial cell may be determined in the present methods. A Chlamydial cell may be a cell from a species belonging to the Chlamydia psittaci group. The Chlamydia psittaci group includes Chlamydia psittaci and Chlamydia pneumoniae.

The binding of antibodies to a Chlamydia antigen may be determined by any appropriate means or assay format. Tagging with individual reporter molecules is one possibility. For example, a second antibody which binds to antibodies in the sample, or a Chlamydia cell or antigen may be tagged with a reporter molecule. The reporter molecules may directly or indirectly generate detectable, preferably measurable, signals. Where required, linkage of reporter molecules may be direct or indirect, covalent, e.g. via a peptide bond, or non-covalent. Linkage via a peptide bond may be as a result of recombinant expression of a gene fusion encoding binding molecule (e.g. antibody) and reporter molecule.

Reporters include fluorochromes such as fluorescein, rhodamine, phycoerythrin and Texas Red, chromogenic dyes such as diaminobenzidine, macromolecular colloidal particles or particulate material such as latex beads that are coloured, magnetic or paramagnetic, and biologically or chemically

active agents that can directly or indirectly cause detectable signals to be visually observed, electronically detected or otherwise recorded.

Biologically or chemically active agents include enzymes which catalyse reactions that develop or change colours or cause changes in electrical properties, for example. They may be molecularly excitable, such that electronic transitions between energy states result in characteristic spectral absorptions or emissions. They may include chemical entities used in conjunction with biosensors. Biotin/avidin or biotin/streptavidin and alkaline phosphatase detection systems may be employed. Further examples include horseradish peroxidase and chemiluminescence. Any such method may be used to determine the binding of the antibody to Chlamydia antigen.

The signals generated by individual antibody-reporter conjugates may be used to derive quantifiable absolute or relative data of the relevant antibody binding in samples (normal and test).

Immunological assays are well-known in the art and many suitable formats are available, for example ELISA, Western blotting, microimmunofluorescence (MIF), Biacore®, (Biacore, Upsala, Sweden), immunoprecipitation or immuno-turbidimetry, agglutination, for example erythrocyte-, latex- or other polymer-based agglutination, immunohistochemistry, immunoelectrophoresis, antibody-based affinity chromatography, IDEIA® (Boots-Celltech) and other red-ox amplifying diagnostic systems.

In some preferred embodiments, a sandwich assay format may be employed. For example, sandwich assay may employ a capture antibody which binds Chlamydia antigen in the sample and a second labelled anti-Chlamydia antibody which detects the

presence of antigen bound to the capture antibody. Alternatively, a sandwich assay may employ a capture Chlamydia antigen or cell and a labelled antibody, for example a labelled anti-human IgG antibody, which detects the presence of anti-Chlamydia antibodies bound to the antigen.

A capture antibody, Chlamydia antigen or Chlamydia cell may be immobilised, for example, by attachment to an insoluble support or solid surface. The support may be in particulate or solid form and may include a plate, a test tube, beads, a ball, a filter or a membrane. Methods for fixing antibodies to insoluble supports are known to those skilled in the art. An antibody may be immobilised, for example, to isolate endogenous antibodies from the sample.

A non-immobilised component of an assay (i.e. a component which is free in solution) such as an antibody or Chlamydia antigen or cell may comprise a detectable label as described above. For example, the antibody may be labelled with a fluorophore such as FITC or rhodamine, a radioisotope, or a non-isotopic labeling reagent such as biotin or digoxigenin; components containing biotin may be detected using "detection reagents" such as avidin conjugated to any desirable label such as a fluorochrome.

The mode of determining binding is not a feature of the present invention and those skilled in the art are able to choose a suitable mode according to their preference and general knowledge.

Lipid oxidation activity of a sample may be antibody-mediated lipid oxidation activity, for example oxidation which is catalysed by abzymes (see, for example, WO03/017992, WO03/019196 and WO03/019198), in particular anti-Chlamydia abzymes. Abzymes may include anti-Chlamydia IgG molecules

which oxidize lipids. The oxidation of plasma lipoproteins by abzymes is a known risk factor, for example, in the development of conditions such as atherosclerosis.

Any suitable physical or chemical treatment may be used to reduce or abrogate lipid oxidation activity in the sample. In some embodiments, the sample may be physically treated to reduce or abrogate lipid oxidation.

For example, the sample may be heated. Preferably, the sample is heated in accordance with any temperature regimen that inactivates lipid oxidation activity but does not affect binding properties of specific binding pair members, i.e. antibodies or antigens, in the sample. A suitable temperature regimen may include heating the sample to 70°C for at least 1, at least 2, at least 3, at least 5, or at least 10 minutes; heating the sample to 56°C for at least 15, at least 20, at least 30, at least 45, or at least 60 minutes; or heating the sample to 37°C for at least 8 hours, at least 12 hours, at least 24 hours, or at least 48 hours.

Other physical treatments may be used to inactivate lipid oxidation activity without affecting the binding properties of specific binding pair members.

The sample may be subjected to repetitive freeze-thaw cycles, for example two or more cycles of freezing followed by thawing. The sample may be subjected to prolonged storage, for example at least 4 days at 0°C to 4°C, at least 2 or at least 3 months at -10° or at least 4 or at least 6 months at -20°C. The sample may be subjected to high-energy ultrasound, microwave, UV, gamma radiation or any other electro magnetic waves.

The suitability of a treatment or regimen may be determined by treating a sample and measuring the lipid oxidation activity and Chlamydia-binding antibody content of the sample after treatment, as described herein. A suitable treatment or regimen for use in the present methods inactivates lipid oxidation activity but does not affect the binding properties of anti-Chlamydia antibodies or Chlamydia antigens.

In other embodiments, the sample may be chemically treated to inactivate lipid oxidation activity. For example, the sample may be treated with one or more abzyme inactivating agents.

Inactivating agents may include low pH antioxidants (i.e. inhibits oxidation reactions at pH5.5), hydroxyl radical scavengers, 'electron trappers' such as crown ethers and steroids, 'electron cushions' such as polyvinyl-based polymers, 'electron sinks', such as ubiquinones and Q_8, copper chelators and calcium chelators.

Examples of suitable inactivating agents include ascorbic acid, acetyl salicylic acid, sodium azide, catechins, including catechin gallate, DMSO, azithromycin, haemoglobin, telithromycin ketek, or derivatives, analogues and salts of any of these.

In other embodiments, an inactivating agent may be a bacterial cell, for example a cell from probiotic bacteria such as lactobacilli, or a product of such a cell.

The efficacy of a treatment in inactivating lipid oxidation activity in sample may be determined by measuring the lipid oxidation activity of abzymes, for example IgG obtained from a patient atheroma, before and after being subjected to the treatment. Any convenient method of determining lipid oxidation may be used. Many methods for determining lipid

oxidation are known in the art and may be used to determine the reduction or abrogation of lipid oxidation activity in a sample. Suitable methods are, for example, described in CRC Handbook of Methods for Oxygen Radical Research, CRC Press, Boca Raton, Florida (1985), Oxygen Radicals in Biological Systems. Methods in Enzymology, v. 186, Academic Press, London (1990); Oxygen Radicals in Biological Systems. Methods in Enzymology, v. 234, Academic Press, San Diego, New York, Boston, London (1994); and Free Radicals. A practical approach. IRL Press, Oxford, New York, Tokyo (1996) In preferred embodiments, oxidation is determined by determining the production (i.e. the presence or amount) of a lipid oxidation product, which may include aldehydes such as malondialdehyde (MDA), (lipid) peroxides, diene conjugates or hydrocarbon gases.

Another aspect of the invention provides a method of preparing a sample for measurement of anti-Chlamydia antibody or Chlamydia antigen levels in the sample, the method comprising;

reducing or abrogating lipid oxidation activity in said sample.

Following the reduction or abrogation of lipid oxidation activity, the levels of anti-Chlamydia antibody or Chlamydia antigen in the sample may be measured using conventional immunoassay techniques.

The sample from the individual may be further treated to inhibit or reduce complement activity. In some embodiments, the sample may be treated with a complement inhibitor. Inhibitors of complement activity are well known in the art and include, for example, Ca^{2+} chelators such as EGTA or EDTA, thymidine kinase inhibitors, including catechins such as epigallocatechin gallate (EGCG), polysaccarides such as zymosan, peptidyl molecules such as CD46, CD55, CD59,

pexelizumab, eculizumab, compstatin, Cobra venom, antibodies against C1q and other components or intermediates of complement cascade, and fragments of these antibodies, and compounds which imitate the functions and properties of the complement cascade.

In other embodiments, the sample may be treated with a procedure or regimen which inhibits complement activity. Suitable procedures include heating the sample, for example to 56°C for 30 minutes, or 70°C for 2-5 minutes, or other temperature regimen that inactivates complement. Other physical procedures, such as ultrasound shock, irradiation and/or laser treatment, may also be used.

Various further aspects and embodiments of the present invention will be apparent to those skilled in the art in view of the present disclosure. All documents mentioned in this specification are incorporated herein by reference in their entirety.

The invention encompasses each and every combination and sub-combination of the features that are described above.

Certain aspects and embodiments of the invention will now be illustrated by way of example and with reference to the figures described above and tables described below.

Figure 1 shows an assay of Chlamydia antigen(s) levels in serum of CHD patients by ELISA as described herein. Dark columns are aliquots of serum where abzymes were inactivated, light columns are untreated aliquots. *Chlamydia* antigen was detected in concentrations of more than 1 μg per ml of serum in 14 out of 21 samples from patients with CHD. 0.1 E_{450nm} = 2 μg of Chlamydia LPS, starting from the Cut-off level of 0.2 E_{450nm}

Figure 2 shows a comparison of lipid oxidation and Chlamydia antigen damage assays to measure the activity of anti-*Chlamydia* abzymes.

Table 1 shows a comparison of antibody measurement by ELISA in samples of patient sera, in healthy individuals, individuals with respiratory disorders and individuals with Coronary Heart Disease & Cerebral Atherosclerosis, where some of them were treated for abzyme inactivation but the others remained untreated.

Table 2 shows a comparison of antibody measurement by ELISA in treated and untreated samples of patient sera, in healthy individuals, individuals with respiratory disorders and individuals with Coronary Heart Disease & Cerebral Atherosclerosis.

Table 3 shows the effect of lipid oxidation inactivation on the results of MIF testing on anti-*Chlamydia pneumonia** antibodies IgG in human sera.

Table 4 shows the activity of various compounds in inactivating antibody mediated lipid oxidation activity.

Examples

Materials and Methods

Preparation of Samples

Blood samples were obtained from healthy individuals, individuals suffering from respiratory disorders and individuals with coronary heart disease & cerebral atherosclerosis.

Serum was isolated by centrifugation and stored at -20° C for not more than three months. After thawing, samples were tested either immediately or within 24 hours.

Inactivation of Lipid Oxidation Activity

For physical inactivation, the same sample was split into two aliquots. One aliquot was heated in a water bath for 30 minutes at 56°C. The other was untreated. Following treatment of the first aliquot, both samples are taken and tested in the same fashion.

For chemical inactivation, a diluent solution was divided into two portions. In one portion, an abzyme inhibitor was added. The following abzyme inhibitors were used DMSO, 0.1-10%; sodium azide, 10^{-5}-10^{-3}M; catechins, 10^{-6}-10^{-3}M; ketek, 10^{-6}-10^{-3}M; lactobacilli culture, 1μM-1mM; ascorbic acid, 10^{-4}-10^{-3}M; acetyl salicylic acid, 10^{-4}-10^{-3}M.

The serum sample was split in two aliquots and one aliquot was diluted by solution containing the abzyme inhibitor, the second aliquot was diluted by the control solution. The aliquots were then tested in the same fashion.

ELISA Assay

ELISA assays were performed using Medac materials and reagents, which were used in accordance with the manufacturers instructions.

Briefly, serum samples from patients were treated as described above. 50 μl of sample diluent was pipetted into microtitre well A1 as blank, and 50 μl of the negative control, Positive Control and the diluted patients' samples were pipetted into other microtitre wells. The microplate wells were incubated for 60 min (± 5 min) at 37°C (± 1°C) in a humid chamber and then washed three times with 200μl wash buffer per well. 50 μl

of Conjugate was then added to each well and the microplate wells incubated again for 60 min (± 5 min) at 37°C (± 1°C) in a humid chamber and then washed. 50 µl of TMB-Substrate, was added to each well and the microplate wells incubated for 30 min (± 2 min) at 37°C (± 1°C) in a humid chamber. The reaction was stopped by adding 100 µl of Stop Solution, to each well.

Photometric reading was performed using a plate reader at 450 nm (ref. 620 - 650 nm) within 15 min after adding the Stop Solution.

To calculate the results, the OD value of the blank (well A1) was subtracted from all other OD values. Preferably, the OD value of the blank was < 0.150, the mean OD value of the Negative Control was < 0.100 and the OD value of the Positive Control was > 0.800. Cut-off = mean OD value of the Negative Control + 0.380. Grey zone = Cut-off ± 10%

MIF assay

Slides with antigens of *Chlamydia trachomatis*, *C. psittaci*, and *C. pneumoniae* were prepared by applying purified elementary bodies of these bacteria.

Sera were diluted to a titer of 1:1024 in phosphate-buffered saline (PBS) and incubated for 30 min at 37°C. After washing in PBS, anti-human IgG, IgA, IgM conjugates were added to the samples. After 30 mins of incubation at 37 C and being washed in PBS, the slide was covered with a cover slip with mounting medium. A fluorescent microscope was used for the reading of the slides. A positive reaction is represented by a "starry sky" appearance: fluorescent green spots on a slightly red background. All samples were evaluated by two independent experts.

Electron microscopy on lysed Chlamydia

Bacteria cells were fixed for 1 hour in 2,5 % solution of glutaraldehyde, made in 0,2 M cacodylic buffer pH 7,2, after that in chrome-osmium solution for another hour. After that, samples were dehydrated in a gradient increase of ethanol and absolute acetone and imbedded in Eponate 12T14 - Araldite 502. Ultra-thin slides were made by using Ultracut Reichert - Jung, stained by 1 % water solution of uranyl acetate and lead citrate.

Slides were examined and photographed using an electron microscope JEM 100C x (with magnification of) x 5300-53000 times.

SDS-PAGE

Polyacrylamide gel electrophoresis was performed using various commercially available systems, in accordance with the manufacturer's instructions. For example, the method described in DPO 033/02; Issue 1.0 "Protein electrophoresis using NOVEX™ system (SDS-PAGE)" was used withthe following reagents: NuPAGE™ Bis-Tris 4-12% precast gels (Invitrogen NP0321 batch #2063076) (15 well); NuPAGE™ Bis-Tris 4-12% precast gels (Invitrogen NP0321 batch #2072272) (10 well); NuPAGE™LDS sample buffer 4x (Invitrogen NP0007 batch #300277); NuPAGE™ Sample reducing agent x10 (Invitrogen NP0004 batch #300505) NuPAGE™ MOPS SDS running buffer x20 (Invitrogen NP0001 batch #300704); SeeBlue™ pre stained markers (Invitrogen LC5625 batch #see11214).

Determination of peroxidation of lipids

Lipid peroxidation was assessed as a level of MDA concentration which was measured by spectrophotometric method [Draper, H.H. et al Free Radic. Biol. Med. (1993) 15, 353]. This method is based on the formation of a coloured product when malondialdehyde reacts with thiobarbituric acid.

Briefly, the level of abzymes in a sample was determined as follows: Samples of sera were diluted 1:1 by 0.05M acetate buffer pH 4.0 to make the final pH of these samples between 5.6-5.8. 990µl of the diluted serum was mixed with 10µl of the commercial live ovine *Chlamydia* vaccine (Intervet). Samples were incubated overnight (12-16 hours) at 37°C. 250µl of 40% trichloroacetic acid and 250µl of 1mM 2-thiobarbituric acid was added to each sample. All samples were placed in a water bath and boiled for 30 minutes. Samples were cooled down and centrifuged at 3,000g for 10 minutes. The supernatants were collected and their absorption measured at λ 525nm to determine the concentration of malondialdehydes (MDA), which are products of lipid peroxidation.

Results

Antibody Tests

ELISA

Antibodies were measured by ELISA as described above in samples of patient sera. The results are shown in Tables 1 and 2.

These results show that inhibition of the abzymes increased antigen binding in some of the positive samples in the control group, but did not affect antigen binding significantly in the sera of the patients with respiratory diseases.

However, the abzyme inactivation made a significant impact on the detection of the specific anti-*Chlamydia* antibodies in the serum of the patients with clinical complications of atherosclerosis. A significant activity of anti-Chlamydia abzymes can usually be detected there.

Inactivation of the abzymes was accompanied by shifting of the results from the "grey zone" level, or "mildly positive", for the overwhelming majority of the tested samples, to "strongly

positive" reading with absorption of more than 1.0. If, before such treatment, the percentage of strongly positive serum samples was 18%, after abzyme inactivation it became 61%.

MIF

A similar effect of the abzyme inactivation on the measurement of specific anti-*Chlamydia* antibodies was observed in the micro-immunifluorescent assay, MIF (Table 3).

These results indicate that inactivation of abzymes can prevent damage of the antigen(s) used in immunological assays such as ELISA or MIF and thus provide a more accurate measurement of the level of specific antibodies present in analysed samples.

Antigen Tests

ELISA

Results of the measurement of *Chlamydia* antigens in the serum of patients with Coronary Heart Disease by ELISA, using mouse monoclonal anti-*Chlamydia* LPS antibodies conjugated with Horse Radish Peroxidase, are presented in Figure 1.

These results show that if serum sample abzymes were not inactivated, only 3 out of 21 samples were positive on the detection of the *Chlamydia* antigens. However, if samples were pre-treated and the abzymes were inactivated, the number of positives became 14.

These results indicate that inactivation of abzymes can prevent damage of the antigen(s) and improve its detection and/or recovery in immunological, immunochemical, immunohistological or other assays and thus provide a more accurate measurement of the level of antigen present in analysed samples.

Patient Groups	IgG ELISA, in E_{450nm}× 1,000		Difference in absorption
	untreated sera	treated sera	
Control	71	79	8
	90	83	0
	109	104	0
	134	150	16
	177	171	0
	184	247	63
	216	186	0
	272	234	0
	420	400	0
	478	521	43
	517	554	37
	559	590	31
	713	**1069**	**356**
	913	**1150**	**237**
Respiratory Disease	61	97	36
	174	143	0
	174	156	0
	221	195	0
	226	211	0
	236	111	0
	272	363	91
	360	381	21
	416	461	45
	815	667	0
	985	924	0
	1450	**1416**	0
	1561	**1586**	25
Coronary Heart Disease or Cerebral Atherosclerosis	73	87	14
	194	233	39
	301	725	**424**
	365	544	**179**
	446	**1010**	**564**
	478	771	**293**

	538	1263	725
	567	1127	560
	654	1713	1059
	692	617	0
	764	1573	409
	780	1078	298
	781	1325	544
	805	1021	216
	897	1542	645
	988	1291	303
	1022	1528	506
	1175	1227	52

Table 1

Laboratories	Patient Groups					
	Control		Respiratory Diseases		Coronary Heart Disease & Cerebral Atherosclerosis	
Lab R	Serum samples		Serum samples		Serum samples	
	untreated	treated	untreated	treated	untreated	treated
	5 (+) **4 > 1.0** 21%	**5(+)** **4.0 > 1.0** 21%	**2 (+)** **2 > 1.0** 25%	**2 (+)** **2 > 1.0** 25%	**39 (+)** **13 > 1.0** 24%	**47 (+)** **41 > 1.0** 76%
	2 greys 12 (-)	***2 greys*** 12 (-)	***1 grey*** 6 (-)	***0 greys*** 7 (-)	***11 greys*** 4 (-)	***4 greys*** 3 (-)
	n = 19		n = 8		n = 54	
Clinical Lab	**3 (+)** **0 >1.0** 0%	**4 (+)** **0 > 1.0** 0%			**23 (+)** **2 > 1.0** 8.7%	**27 (+)** **11 > 1.0** 41%
	0 greys 9 (-)	***2 greys*** 6 (-)			***5 greys*** 4(-)	***4 greys*** 1 (-)
	n = 12				n = 32	
CTL Lab	**1 (+)** **0 > 1.0** 0%	**2 (+)** **0 > 1.0** 0%	**6 (+)** **3 > 1.0** 19%	**6 (+)** **3 > 1.0** 19%	**45 (+)** **13 > 1.0** 19%	**55 (+)** **43 > 1.0** 61%
	3 greys 10 (-)	***2 greys*** 10 (-)	***9 greys*** 1 (-)	***5 greys*** 5 (-)	***14 greys*** 11 (-)	***5 greys*** 10 (-)
	n = 14		n = 16		n = 70	
Total	**9 (+)** 20% **4 > 1.0** **8.9%**	**11 (+)** 24% **4 > 1.0** **8.9%**	**8 (+)** 32% **5 > 1.0** **20%**	**8 (+)** 32% **5 > 1.0** **20%**	**107 (+)** 67% **28 > 1.0** **18%**	**129 (+)** 82% **95 > 1.0** **61%**
	5 greys	***6 greys***	***10 greys***	***5 greys***	***31 greys***	***14 greys***
	31 (-)	28 (-)	7 (-)	12 (-)	19 (-)	14 (-)
	n = 45		n = 25		n = 157	

Lab R and Clinical Lab are collaborating with CTL laboratories.

Table 2

Serum ID	MIF test, in titers		
	Untreated serum samples (abzymes detected)	Treated serum samples (abzymes non-detected)	
Abzyme positive			
285 GAZ	32/64	<	512/512
286 TGB	32/64	≤	64/64
288 VPK	16/32	≤	64/64
297 NEC	32/64	<	128/128
302 IVK	16/32	<	64/64
305 VNX	16/32	<	64/64
P577	0	<	1/128
OAG	0	<	1/64
YIO	0	<	1/64
IVM	0	<	1/64
IMK	0-1/16	<	1/64
P580	0	<	1/64
P573	0	<	1/64
P571	0	<	1/32
AFP	0	<	1/16-1/32
VAM	0	<	1/16
P572	0	=	0
Abzyme-negative			
282 AVS	32/32	<	64/64
287 VAG	32/64	≤	64/64
289 VPK	256/1024	≤	1024/1024
292 VLC	64/128	<	256/512
294 YIX	32/32	=	32/32
298 VAP	0/0	=	0/0
P585	0	=	0
AIS	0	=	0
GPM	0	=	0
P567	0	=	0
INK	0	=	0-1/16
SII	0	=	0-1/16
NEC	0	=	0
JON	0	=	0-1/16
KAT	0	=	0
JIM	0	=	0

*Purified Iol and Kajaani 6 strains of *Chlamydia pneumonia* were used

Table 3.

Factors affecting abzyme activity	Inhibition of abzymes ability to cause:	
	Serum lipid peroxidation, in MDA assay	Damage of *Chlamydia pneumoniae* antigen, in ELISA
Physical procedures		
Repetitive freezing thawing	**Positive**	**Positive**
Heating at 56°C for 30 min	**Positive**	**Positive**
Drugs, reagents or food products		
1. Acetyl salicylic acid	**Positive**	**Positive**
2. Ascorbic acid	**Positive**	**Positive**
3. EDTA	**Positive**	**Positive**
4. EGTA	n/a	**Positive**
5. Sodium cyanide	Negative	Negative
6. Sodium azide	**Positive**	**Positive**
7. (+) Catechin gallate	**Positive**	**Positive**
8. ß-Carotene	Negative	Negative
9. (+) α-Tocopherol	Negative	Negative
10. (+) y-Tocopherol	Negative	Negative
11. Benzoic acid	Negative	Negative
12. DMSO	**Positive**	**Positive**
13. D-Mannitol	Negative	Negative
14. PMS	Negative	Negative
15. Haemoglobin	**Positive**	**Positive**
16. Telithromycin, Ketek	**Positive**	**Positive**
17. Tetracycline	Negative	Negative
18. Lactobacilli culture	**Positive**	**Positive**
19. Lycopene	Negative	Negative

* antibody-antigen reaction was blocked by lowered pH after addition of these acid compounds.

Table 4

Claims:

1. A method of measuring the amount of a first Chlamydia binding pair member in a sample comprising;

abrogating lipid oxidation activity in said sample, and

determining the binding of a first Chlamydia binding pair member in the treated sample to a second Chlamydia binding pair member,

the amount of binding after said treatment being indicative of the level of the first Chlamydia binding pair member in the sample.

2. A method according to claim 1 wherein the level of the first Chlamydia binding pair member in the sample is indicative of Chlamydia infection in said individual.

3. A method according to any one of the preceding claims wherein the first Chlamydia binding pair member is a Chlamydia antigen and the second Chlamydia binding pair member is an anti-Chlamydia antibody.

4. A method according to any one of claims 1 to 2 wherein the first Chlamydia binding pair member is anti-Chlamydia antibody and the second Chlamydia binding pair member is a Chlamydia antigen.

5. A method according to any one of the preceding claims wherein the sample is a serum sample obtained from an individual.

6. A method according to any one of the preceding claims wherein the sample is physically treated to inactivate lipid oxidation activity.

7. A method according to claim 6 wherein the sample is heated.

8. A method according to claim 7 wherein the sample is heated to at least 37°C for at least 5 minutes.

9. A method according to claim 8 wherein the sample is heated to 56°C for 30 mins.

10. A method according to claim 6 wherein the sample is exposed to two or more freeze thaw cycles.

11. A method according to claim 6 wherein the sample is maintained at 0°C to 4°C for 4-7 days.

12. A method according to any one of claims 1 to 5 wherein the sample is chemically treated to inactivate abzymes.

13. A method according to claim 12 wherein the sample is treated with one or more inactivating agents.

14. A method according to claim 13 wherein the inactivating agent is a hydroxyl radical scavenger, low pH anti-oxidant, electron trapper, cushion or sink.

15. A method according to any one of claims 12 to 14 wherein the inactivating agent is selected from the group consisting of ascorbic acid, acetyl salicylic acid, sodium azide, (+) catechin gallate, DMSO, haemoglobin and telithromycin ketek or analogues or derivatives thereof.

16. A method according to claim 13 wherein the inactivating agent is a bacterial cell

17. A method according to claim 16 wherein the inactivating agent is a lactobacillus cell.

18. A method according to any one of the preceding claims wherein the abzyme mediated lipid oxidation of said sample is determined following said abrogation.

19. A method according to any one of the preceding claims wherein the sample is further treated to reduce or abrogate complement activity.

20. A method according to any one of the preceding claims wherein the second Chlamydia binding pair member is immobilised.

21. A method according to any one of claims 1 to 19 wherein the second Chlamydia binding pair member is labelled.

22. A method according to claim 21 wherein the second Chlamydia binding pair member is labelled with a detectable reporter molecule.

23. A method according to any one of the preceding claims wherein the Chlamydia antigen is on the surface of a Chlamydia cell.

24. A method according to any one of the preceding claims wherein the binding of the second Chlamydia binding pair member to the first Chlamydia binding pair member is determined using a second antibody.

25. A method according to claim 24 wherein the second antibody is an anti-Chlamydia antibody or an anti-IgG antibody.

26. A method according to claim 24 or claim 25 wherein the second antibody is labelled.

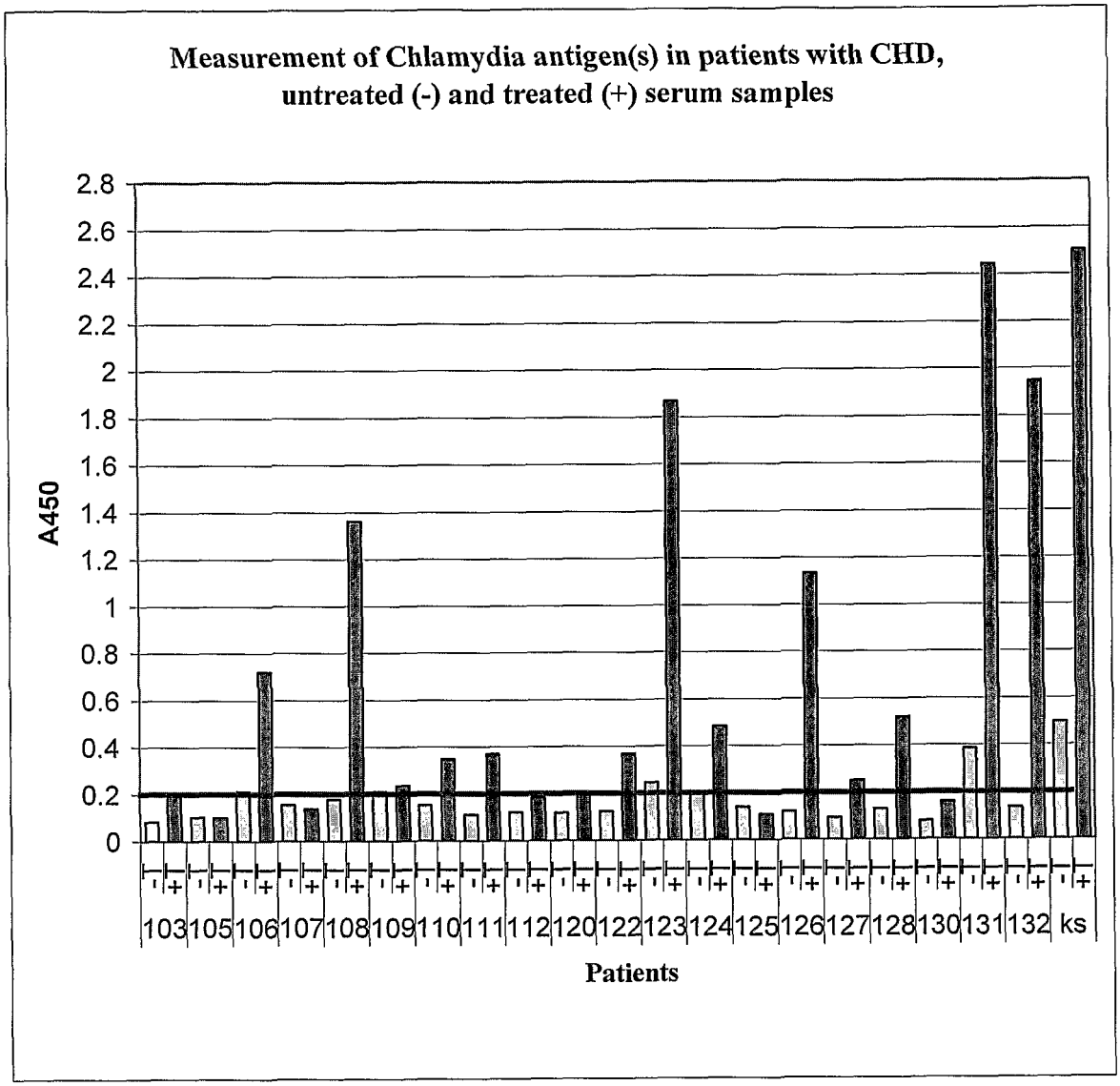
Measurement of Chlamydia antigen(s) in patients with CHD, untreated (-) and treated (+) serum samples
A450
2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
103 105 106 107 108 109 110 111 112 120 122 123 124 125 126 127 128 130 131 132 ks
Patients

Figure 1

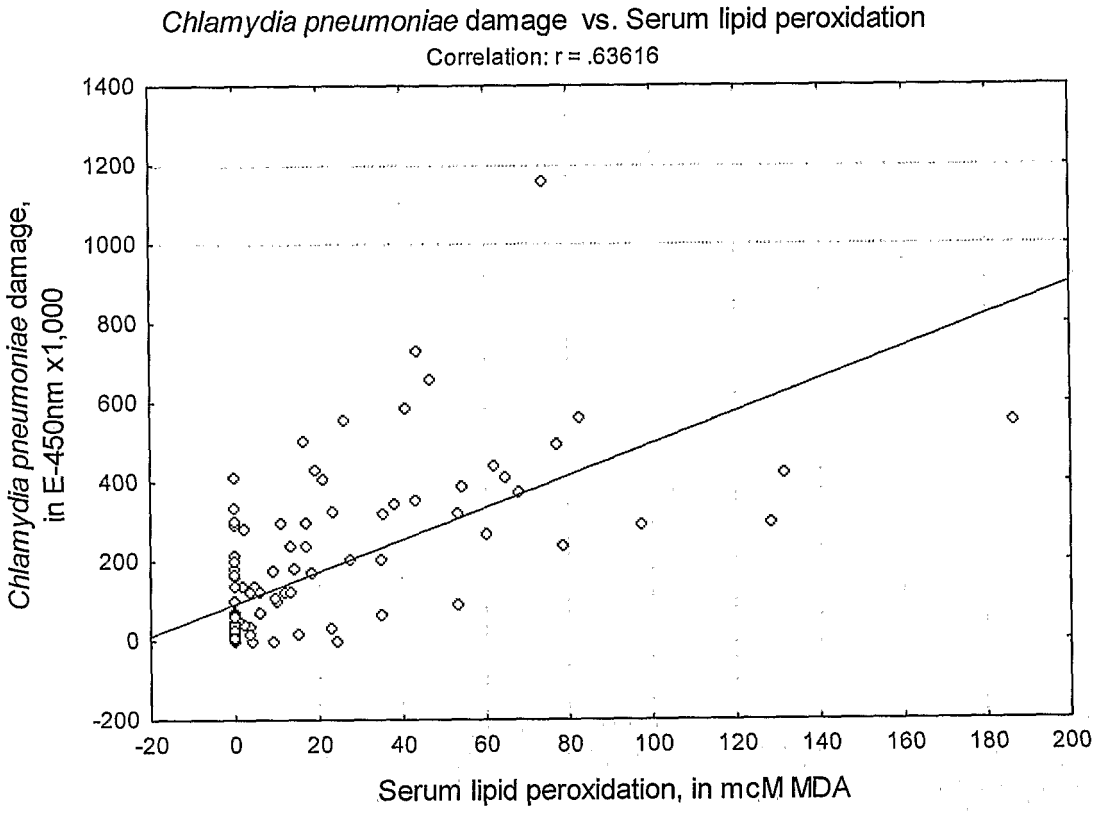
Chlamydia pneumoniae damage vs. Serum lipid peroxidation
Correlation: r = .63616
Chlamydia pneumoniae damage, in E-450nm x1,000
1400
1200
1000
800
600
400
200
0
-200
-20
0
20
40
60
80
100
120
140
160
180
200
Serum lipid peroxidation, in mcM MDA

Figure 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2006/000655

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/53 G01N33/569 G01N33/573

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)
G01N

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 practical, search terms used)
EPO-Internal, WPI Data, PAJ, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/017992 A (CAMBRIDGE THERANOSTICS LTD; PETYAEV, IVAN) 6 March 2003 (2003-03-06) page 8, paragraph 3 - page 14, paragraph 8 page 9, paragraph 5 - page 10, paragraph 1 page 17, paragraph 1-4 page 20, paragraph 4 - page 21, paragraph 4 page 22, paragraph 4 page 56, paragraph 4 tables 7,9,11,13,14,16,23,24 example 1 figure 1 ----- -/--	1-26

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 April 2006	18/05/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Graf, E.M.

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	"Labor und Diagnose: Indikation und Bewertung von Laborbefunden für die medizinische Diagnostik" 7 October 2003 (2003-10-07), LOTHAR THOMAS , DE, FRANKFURT AM MAIN , XP002377086 ISBN: 3-9805215-3-2 page 1211 -----	1-26

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2006/000655

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 03017992 A	06-03-2003	EP 1454140 A2	08-09-2004
		EP 1456402 A2	15-09-2004
		EP 1456670 A2	15-09-2004
		WO 03019198 A2	06-03-2003
		WO 03019196 A2	06-03-2003
		JP 2005502665 T	27-01-2005
		JP 2005501258 T	13-01-2005

专利名称(译)	检测样品中衣原体的方法		
公开(公告)号	EP1859275A1	公开(公告)日	2007-11-28
申请号	EP2006709887	申请日	2006-02-24
申请(专利权)人(译)	CAMBRIDGE治疗诊断有限公司		
当前申请(专利权)人(译)	CAMBRIDGE治疗诊断有限公司		
发明人	PETYAEV, IVAN CAMBRIDGE THERANOSTICS LIMITED		
IPC分类号	G01N33/53 G01N33/569 G01N33/573		
CPC分类号	G01N33/56927 G01N33/5306		
优先权	2005003939 2005-02-25 GB		
其他公开文献	EP1859275B1		
外部链接	Espacenet		

摘要(译)

用于检测样品中衣原体的抽象方法本发明涉及衣原体的测定，其包括使生物样品（例如血液或血清样品）中的脂质氧化活性失活的步骤。这种失活改善了衣原体抗原或抗衣原体抗体的检测。提供了用于检测衣原体和衣原体感染的方法和材料。