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(54) **Title:** COMPOSITIONS AND METHODS FOR THE DETECTION OF ANAPLASMA PLATYS

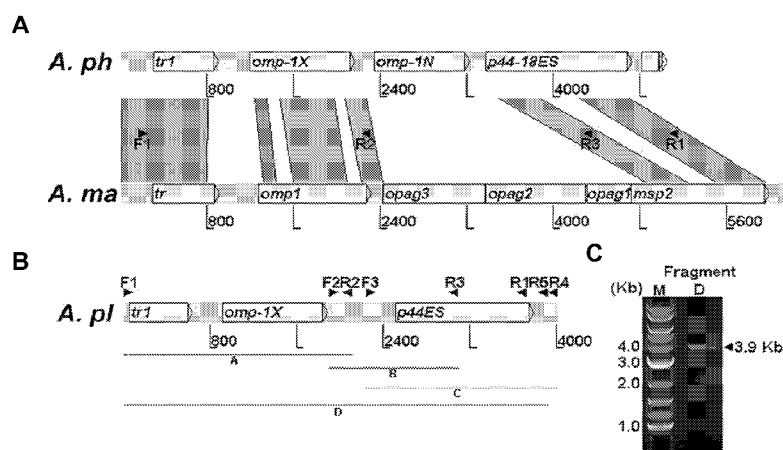


Figure 1

(57) **Abstract:** Described herein are improved diagnostic tools for veterinary and human use which can be used for serodiagnosing *A. platys* in mammals, particularly in members of the Canidae family and in humans. The diagnostic tools are a group of outer membrane proteins of *A. platys* and variants thereof, referred to hereinafter as the "OMP proteins", a group of outer membrane proteins of *A. platys* and variants thereof referred to hereinafter as the "P44 proteins", and antibodies to the OMP proteins and the P44 proteins.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/31580

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07H 21/04, C12Q 1/68 (2012.01)

USPC - 536/24.33, 435/5.12

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)
USPC - 536/24.33, 435/5.12, 536/24.3, 536/23.1, 435/6.1, 435/6.11

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST -- PGPB,USPT,USOC,EPAB,JPAB; Dialog Classic Files -- 654, 652, 349, 348, 340, 35, 65, 155; USPTO Web Page; Google Scholar; EMBL DBFetch; Search terms -- detection, forward/reverse primers, Anaplasma platys, p44, amplification, PCR, vector, recombinant, PCR

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2009/0155825 A1 (BEALL et al.) 18 June 2009 (18.06.2009) para [0007], [0009], [0011]-[0013], [0018], [0039], [0043]-[0045], [0080], [0083], Fig 1, abstract	36 --- 1, 2, 4, 5, 10, 11
X -- Y	EMBL Accession Number DQ363749, Anaplasma centrale RecJ gene, partial cds. 4219 bps sequence, 12 February 2006 (12.02.2006), retrieved from the internet <http://www.ebi.ac.uk/Tools/dbfetch/dbfetch?db=embl&id=DQ363749&f.> 10 September 2012, pg 2-3	35 --- 1, 2, 4, 5, 10, 11
Y	EMBL Accession Number AY040556, Anaplasma centrale clone 337 major surface protein-2 gene, complete cds. 1203 bps sequence, 5 February 2002 (05.02.2002), retrieved from the internet <http://www.ebi.ac.uk/Tools/dbfetch/dbfetch?db=embl&id=AY040556&f.> 10 September 2012, pg 2-3	1, 2, 4, 5, 10, 11

☐ Further documents are listed in the continuation of Box C. ☐

* 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 application or patent 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

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/31580

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 20, 23, 37-39, 53, 56-67, 72, 77, 79 and 81-85
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+ claims 1-6, 10, and 11, 35 and 36 directed to a method for detecting *Anaplasma platys* in a sample obtained from a subject, an isolated polynucleotide and a vector for transformation of a host cell comprising (a) providing a primer set comprising: (i) one or more forward primers comprising the sequence of: SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, or SEQ ID NO: 90 and (ii) one or more reverse primers comprising the sequence of: SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89 or SEQ ID NO: 91.

SEE CONTINUATION SHEET.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1, 2, 4, 5, 10, 11, 35 and 36, restricted to SEQ ID NOs: 82 and 85

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Continuation of Box III

Group II+ claims 7-9, 35, and 36 directed to a method for detecting *Anaplasma platys* in a sample obtained from a subject, an isolated polynucleotide and a vector for transformation of a host cell comprising (a) providing a primer set comprising: (i) one or more forward primers comprising the sequence of: SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62 and (ii) one or more reverse primers comprising the sequence of: SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, or SEQ ID NO: 63.

Group III+ claims 12-16, 35 and 36 directed to a method for detecting *Anaplasma platys* in a sample obtained from a subject, an isolated polynucleotide and a vector for transformation of a host cell comprising (a) providing a primer set comprising: (i) one or more forward primers comprising the sequence of: SEQ ID NO: 64, SEQ ID NO: 18, or SEQ ID NO: 20 and (ii) one or more reverse primers comprising the sequence of: SEQ ID NO: 65, SEQ ID NO: 19, or SEQ ID NO: 21.

Group IV+ claims 17-19, 21, 22, 24-34, 40-52, 54, 55, and 86-103 directed to an isolated polynucleotide encoding an outer membrane protein of *Anaplasma platys*, or a fragment thereof, wherein the outer membrane protein is P44 or OMP-IX, an isolated polynucleotide encoding a variant of an outer membrane protein of *Anaplasma platys*, or a fragment thereof, wherein the outer membrane protein is P44 or OMP-IX protein and a method of detecting antibodies that specifically bind an *Anaplasma platys* polypeptide, wherein the polynucleotide comprises SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97 and fragments thereof, and wherein the isolated polynucleotide is capable of encoding SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19, the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, and fragments thereof.

Group V claims 68-71, 73-76, 78, and 80 directed to a method and kit for diagnosing an infection with *Anaplasma platys* in a subject comprising the steps of: (a) providing a serum sample from the subject; (b) providing an isolated or purified P44 protein or OMP-IX protein of *Anaplasma platys*; (c) contacting the serum sample with the P44 protein; and (d) assaying for the formation of a complex between antibodies in the serum sample and the P44 protein, wherein formation of said complex is indicative of infection with *Anaplasma platys*.

The inventions listed as Groups I+-V do not relate to a single inventive concept under Rule 13.1 because under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical features of the Groups are delineated above. Groups I+-III+ share the special technical features of a method for detecting *Anaplasma platys* in a sample obtained from a subject, comprising (a) providing a primer set comprising: (i) one or more forward primers and (ii) one or more reverse primers; (b) amplifying DNA in the sample with the said primer set and a polymerase chain reaction, and (c) determining the length or sequence of the PCR products of step (b), wherein the presence of a PCR product having a length or sequence which corresponds to the length or sequence, respectively, of that region of the *Anaplasma platys* p44 gene or OMP-IX gene which is located between the regions to which the one or more forward primers and the one or more reverse primers bind is indicative of the presence of *Anaplasma platys* in the sample and wherein the one or more forward or reverse primers are from 15 to 35 nucleotides in length; an isolated polynucleotide, and a vector for transformation of a host cell, said vector comprising an isolated polynucleotide that encodes an outer membrane protein of *Anaplasma platys*, a variant of said outer membrane protein, or an immunogenic fragment of said outer membrane protein. However, this is not an improvement over the prior art of US 2009/0155825 A1 to Beall et al. (hereinafter Beall) that teaches a method for detecting *Anaplasma platys* in a sample obtained from a subject (abstract), comprising (a) providing a primer set comprising: (i) one or more forward primers and (ii) one or more reverse primers (para [0011], [0082]); (b) amplifying DNA in the sample with the said primer set and a polymerase chain reaction (para [0083]), and (c) determining the length or sequence of the PCR products of step (b), wherein the presence of a PCR product having a length or sequence which corresponds to the length or sequence, respectively, of that region of the *Anaplasma platys* p44 gene (para [0081], [0018], Fig 1A) or OMP-IX gene which is located between the regions to which the one or more forward primers and the one or more reverse primers bind is indicative of the presence of *Anaplasma platys* in the sample (para [0082]-[0083]) wherein the one or more forward or reverse primers are from 15 to 35 nucleotides in length (para [0082]).

SEE CONTINUATION SHEET.

INTERNATIONAL SEARCH REPORT

International application No.

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Continuation of:
Box III, Supplemental Page 1

Further, Beall teaches the primer set comprising the forward primer of SEQ ID NO: 90 (SEQ ID NO: 17 at 100% homology for residues 387-406 of the polynucleotide) and the reverse primer of SEQ ID NO: 91 (SEQ ID NO: 17 at 100% homology for residues 631-610 of the polynucleotide) wherein the generation and use of appropriate primer sets based on the known nucleic acid sequence of a target polynucleotide would have been readily obvious to one of ordinary skill in the art as a standard and well known methodology for amplifying polynucleotides. The Groups and Sub-Groups are different because the different polynucleotide forward primer sequences represented by SEQ ID NOs: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 90, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, or SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 18, and SEQ ID NO: 20 and reverse primer sequences represented by SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 19, and SEQ ID NO: 21 are different structures that are not common to one another but are different because they are composed of unique nucleic acid sequences.

Group IV+ shares with Groups I+-III+ the special technical feature of an isolated polynucleotide encoding an outer membrane protein of *Anaplasma platys*, or a fragment thereof, wherein the outer membrane protein is P44 or OMP-IX, but, this is not an improvement over the prior art of Beall as described hereinabove. Group IV+ further requires a method of detecting antibodies that specifically bind an *Anaplasma platys* polypeptide, wherein the polynucleotide comprises any of several designated polypeptide sequences and the isolated polynucleotide is capable of encoding any of several sequences. This special technical feature is not required by any of the other Groups. (Further Beall specifically teaches a method of detecting antibodies that specifically bind an *Anaplasma platys* polypeptide, wherein the polynucleotide comprises any of several designated polypeptide sequences (para [0006], [0050])). Further, the Sub-Groups are different because the different polynucleotide sequences represented by SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 1, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 39, SEQ ID NO: 42, SEQ ID NO: 40, SEQ ID NO: 93, SEQ ID NO: 41, SEQ ID NO: 94, SEQ ID NO: 42, SEQ ID NO: 95, SEQ ID NO: 43, SEQ ID NO: 96, SEQ ID NO: 44, SEQ ID NO: 97 and fragments thereof are different structures that are not common to one another but are different because they are composed of unique nucleic acid sequences. Further, the polypeptide sequences represented by SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, the amino acid sequence comprising the amino acid from about position 20 to about position 40 of Figure 23, the amino acid sequence comprising the amino acid from about position 40 to about position 64 of Figure 23, the amino acid from about position 75 to about position 85 of Figure 23, the amino acid sequence comprising the amino acid from about position 102 to about position 111 of Figure 23, the amino acid from about position 170 to about position 190 of Figure 23, the amino acid sequence comprising the amino acid from about position 178 to about position 222 of Figure 23, the amino acid from about position 205 to about position 215 of Figure 23, the amino acid sequence comprising the amino acid from about position 259 to about position 266 of Figure 23, the amino acid from about position 270 to about position 290 of Figure 23, the amino acid sequence comprising the amino acid from about position 319 to about position 340 of Figure 23, the amino acid from about position 365 to about position 380 of Figure 23, the amino acid sequence comprising the amino acid from about position 451 to about position 460 of Figure 23, the amino acid from about position 1 to about position 41 of Figure 19, the amino acid sequence comprising the amino acid from about position 78 to about position 85 of Figure 19, the amino acid from about position 174 to about position 192 of Figure 19, the amino acid sequence comprising the amino acid from about position 227 to about position 234 of Figure 19, the amino acid from about position 276 to about position 294 of Figure 19, the amino acid sequence comprising the amino acid from about position 416 to about position 433 of Figure 19, the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, a combination of the amino acid from about position 66 to about position 192 and the amino acid from about position 70 to about position 180 of Figure 22, the amino acid from about position 240 to about position 312 of Figure 22, the amino acid from about position 230 to about position 300 of Figure 22, a combination of the amino acid from about position 240 to about position 312 and the amino acid from about position 230 to about position 300 of Figure 22, and fragments thereof are unique are different structures that are not common to one another but are different because they are composed of unique amino acid sequences.

The special technical features of Group V are (a) providing a serum sample from the subject; (b) providing an isolated or purified P44 protein or OMP-IX protein of *Anaplasma platys*; (c) contacting the serum sample with the P44 protein; and (d) assaying for the formation of a complex between antibodies in the serum sample and the P44 protein, wherein formation of said complex is indicative of infection with *Anaplasma platys*. None of these features is required by the other Groups.

Therefore the inventions listed as Groups I+-V do not relate to a single general inventive concept under PCT Rule 13.1 because they do not share a same or corresponding special technical feature. This ISA will establish the ISR for the first Group mentioned, specifically, Group I claims 1-6, 10, and 11, 35 and 36 restricted to the forward primer represented by SEQ ID NO: 82 and the reverse primer represented by SEQ ID NO: 85 without additional fees. In order for other inventions to be searched from Groups I+-III+, applicants must designate with specificity the particular forward and reverse primer pair corresponding to the appropriate sequences to be elected. In order for any of the inventions to be searched from Group IV+ applicants must designate with specificity the polynucleotide sequence and corresponding polypeptide sequence and further with respect to any particular amino acid variation so desired for that particular pair (ie ?The isolated polynucleotide of claim 28, wherein the polynucleotide encodes the amino acid sequence comprising the amino acid from about position 66 to about position 192 of Figure 22, the amino acid from about position 70 to about position 180 of Figure 22, etc.).