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(54) Title: METHODS AND COMPOSITIONS FOR DETECTING BACTERIAL CONTAMINATION

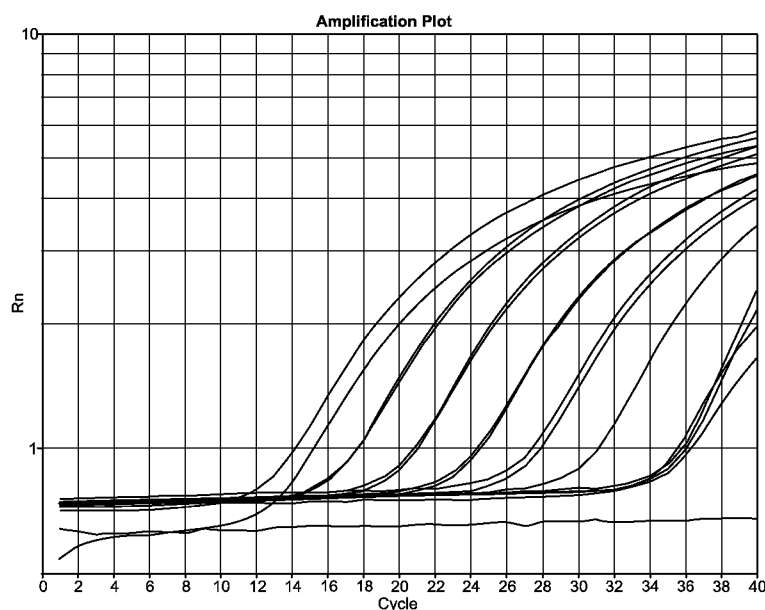


FIG. 1

(57) Abstract: Compositions, reactions
mixtures, kits, and systems for detecting
bacterial contamination are provided, as
well as methods of using the same.



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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 14/47914

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/68 (2014.01)

CPC - C12Q 1/689

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)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC- C12Q 1/6888, C12Q 1/6876Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase (PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD), FreePatentsOnline (US Pat, PgPub, EPO, JPO, WIPO, NPL),
GoogleScholar (PL, NPL); search terms: method composition detect bacterial contamination platelet amplification 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	WO 2001/023604 A2 (BERGERON et al.) 05 April 2001 (05. 04.2001) Abstract, pg 31, para 3, pg 78, Example 12; pg 79, para 1, ln 1-2; pg 99, para 2; pg 118, para 1; pg 135, para 3	1, (9-12)/1, 13, (16-17)/1 ----- (5-6)/1, (18-19)/1
Y	WO 2004/050909 A2 (ABASTADO et al) 17 June 2004 (17.06.2004) Abstract; pg 5, ln 1-4; pg 8,	(5-6)/1
Y	Critical Factors for Successful Real-Time PCR, by Quiagen, Sample & Assay Technologies Brochure, pp 1-64 (07/2010), [online]. [Retrieved on 12 January 2015 (12.01.2015)]. Retrieved from the internet: <URL: http://www.gene-quantification.de/qiagen-qpcr-sample-assay-tech-guide-2010.pdf > Especially pg 15, section 5, Figs 10-11; pg 16, section 5.2, Fig 12	(18-19)/1

☐ 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

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 14/47914

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.:
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 as to form a single general inventive concept under PCT Rule 13.1.
Group I, claims 1, 5-6 (in part), 9-12 (in part), 13, and 16-19 (in part), directed to a method of detecting bacterial contamination of a platelet sample by any of at least eight bacterial species, wherein a primer pair flanks the amplicon, the amplicon comprises a conserved sequence to which the detectable probe hybridizes, and the conserved sequence is identical among the at least eight bacterial species; and wherein a detectable signal is indicative of bacterial contamination of the platelet sample by any of the at least eight bacterial species.
Group II, claims 2, 5-6 (in part), 9-10 (in part), and 17-19 (in part), directed to a method wherein the sample or portion thereof has not been cultured at a temperature above 35 C prior to amplification.
Group III, claims 3, 5-6 (in part), 7, 9-10 (in part), and 17-19 (in part), directed to a method of rapidly detecting bacterial contamination in a platelet sample within 24 hours after obtaining the sample, and detecting hybridization to said amplicon by said probe; thereby detecting the contamination within about 24 hours from obtaining said sample.
-----Continued in the supplemental box-----

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, 5-6, 9-13, 16-19

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 V, claims 22-23 and 30-31 directed to a composition, kit and system for amplification and detection comprising a first primer comprising at least 10 contiguous nucleotides of the sequence as set forth in SEQ ID NO: 9; a second primer comprising at least 10 contiguous nucleotides of the sequence as set forth in SEQ ID NO: 10; and, a probe comprising at least 10 contiguous nucleotides of the sequence as set forth in SEQ ID NO: 16, or the complement thereof; and a computer-readable medium.

Group VI, claims 24-29, directed to a composition for amplification and detection comprising primers that amplify an amplicon of at least 50 nucleotides in length, the amplicon having 90 sequence identity with any of SEQ ID NO: 1-3 when optimally aligned; and a probe that specifically hybridizes to either strand of the amplicon.

Group VII, claims 35-46, directed to a method of detecting bacterial contamination of a platelet sample by any of at least five bacterial species using a reaction mixture comprising a first forward primer and a first reverse primer, wherein the first forward primer and the first reverse primer each hybridizes to a separate region that is conserved amongst at least 5 different bacterial genomes.

The groups of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features (Distinct Technical Features):

The special technical feature of Group I is the detection of at least eight bacterial species, wherein a primer pair flanks the amplicon, the amplicon comprises a conserved sequence to which the detectable probe hybridizes, and the conserved sequence is identical among the at least eight bacterial species, and wherein a detectable signal is indicative of bacterial contamination of the platelet sample by any of the at least eight bacterial species; not required in any other group.

The special technical feature of Group II is a method of detection wherein the sample or portion thereof has not been cultured at a temperature above 35 C prior to amplification; not required in any other group.

The special technical feature of Group III, is the rapid detection of bacterial contamination in a platelet sample within 24 hours after obtaining the sample; and detecting hybridization to said amplicon by said probe; thereby detecting the contamination within about 24 hours from obtaining said sample; not required in any other group.

The special technical feature of Group IV is the detection of a plurality of bacterial species from different genera in a biological sample of a subject, comprising performing nucleic acid amplification on a 16S rRNA polynucleotide, wherein amplification of about 1pg-5pg of DNA from any one of the species has a cycle threshold value (Ct) of less than 30; and detecting the amplicon with one or more detectable probes, wherein each of the one or more detectable probes specifically hybridizes to a conserved sequence, and the conserved sequence is identical among a plurality of bacterial species from different genera; not required in any other group.

The special technical feature of Group V is a first primer comprising at least 10 contiguous nucleotides of the sequence as set forth in SEQ ID NO: 9; a second primer comprising at least 10 contiguous nucleotides of the sequence as set forth in SEQ ID NO: 10 and a probe comprising at least 10 contiguous nucleotides of the sequence as set forth in SEQ ID NO: 16, or the complement thereof; not required in any other group.

The special technical feature of Group VI is a composition comprising primers that amplify an amplicon of at least 50 nucleotides in length, the amplicon having 90 sequence identity with any of SEQ ID NO: 1-3 when optimally aligned; and a probe that specifically hybridizes to either strand of the amplicon; not required in any other group.

The special technical feature of Group VII is the detection of bacterial contamination of a platelet sample by any of at least five bacterial species, using a reaction mixture comprising a first forward primer and a first reverse primer, wherein the first forward primer and the first reverse primer each hybridizes to a separate region that is conserved amongst at least 5 different bacterial genomes; not required in any other group.

Common Technical Features (Features Do Not Make a Contribution Over the Prior Art):

Groups I-VII share the technical feature of primers that amplify an amplicon and a probe that hybridizes to the amplicon for detecting bacterial contamination. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is anticipated by US 2012/0035071 A1 to Bergeron et al. (hereinafter 'Bergeron'). Bergeron discloses primers that amplify an amplicon and a probe that hybridizes to the amplicon for detecting bacterial contamination (para [0270] - "Real-time detection of PCR products using the LightCycler.TM. was carried out using 0.4 .mu.M of each primer (SEQ ID NO. 549-550), 0.2 .mu.M of each probe (SEQ ID NO. 582-583) ... S. agalactiae yielded an amplification signal"; para [0116] - "primers were designed to amplify a group of bacteria consisting of the 17 major bacterial species encountered as contaminants of platelet concentrates ... the primers shown in Example 12 are considered universal for platelet-contaminating bacteria. To develop an assay specific for the latter, one or more primers or probes specific to each species could be designed"). As the technical feature was known in the art at the time of the invention, it cannot be considered a special technical feature that would otherwise unify the groups.

Groups I-IV and VII share the technical feature of a method of detecting bacterial contamination of a platelet, comprising: subjecting the sample or a portion thereof to a nucleic acid amplification reaction under conditions to yield a detectable amount of an amplicon of no more than about 800 bases in a single reaction mixture, said reaction mixture comprising a single primer pair and a single detectable probe, wherein the primer pair flanks the amplicon, the amplicon comprises a conserved sequence to which the detectable probe hybridizes, and detecting hybridization to the amplicon by the probe, wherein the hybridization by the probe yields a detectable signal indicative of bacterial contamination of the platelet sample. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by Bergeron.

---see next page---

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Bergeron discloses a method of detecting bacterial contamination of a platelet sample by any of at least eight bacterial species (para [0287], Example 12: "Oligonucleotide sequences which are conserved in these 17 major bacterial contaminants of platelet concentrates were chosen (oligos Tplaq 769 and Tplaq 991, respectively SEQ ID NOs. 636 and 637) thereby permitting the detection of these bacterial species. ... The bacterial species detected with the assay are listed in Table 14"; para [0289] - "With this assay, all prominent bacterial contaminants of platelet concentrates listed in Annex XIX and Table 14 were detected. Sensitivity tests were performed on the 9 most frequent bacterial contaminants of platelets"), comprising:
subjecting the sample or a portion thereof to a nucleic acid amplification reaction under conditions to yield a detectable amount of an amplicon of no more than about 800 bases (para [0287]; para [0288] - "The primer pairs, oligos SEQ ID NO. 636 and SEQ ID NO. 637 that give an amplification product of 245 pb, and oligos SEQ ID NO. 553 and SEQ ID NO. 575 that give an amplification product of 368 pb, were used simultaneously in the multiplex PCR assay"), the amplicon comprises a conserved sequence to which the detectable probe hybridizes (para [0286]-[0287]), and the conserved sequence is identical among the at least eight bacterial species (para [0287], Example 12: "Oligonucleotide sequences which are conserved in these 17 major bacterial contaminants of platelet concentrates"); and detecting hybridization to the amplicon by the probe, wherein the hybridization by the probe yields a detectable signal indicative of bacterial contamination of the platelet sample by any of the at least eight bacterial species (para [0287] - "The bacterial species detected with the assay are listed in Table 14"; para [0289] - "Fluorogenic detection of PCR products with the LightCycler"). As the technical feature was known in the art at the time of the invention, it cannot be considered a special technical feature that would otherwise unify the groups.

Groups III-IV share the technical feature of amplification of about 1pg-5pg of DNA from any one of the species has a cycle threshold value (Ct) of less than 30 and detecting hybridization to said amplicon by said probe. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is obvious over US 2012/0141990 A1 (Paitan).

Paitan discloses amplification of DNA from any one of the species (para [0007]), has a cycle threshold value (Ct) of less than 30 and detecting hybridization to said amplicon by said probe (para [0215]: Ct value of 25.79), but does not provide specific units 1pg-5pg of DNA. However, it would have been obvious to a person having ordinary skill in the art of molecular biology to measure the amplification of DNA and represent it in a particular units of choice such as pg, and further because it is routine practice to vary the amounts as needed for identification purposes and obtain an optimum workable range of amplification, and further because the Ct values of Paitan are below 30 as that of the feature indicating the amplification is also same or similar, and if measured it would be from about 1pg-5pg of DNA. As the technical feature was known in the art at the time of the invention, it cannot be considered a special technical feature that would otherwise unify the groups.

Groups IV-VI share the technical feature of amplification and detection of a 16S rRNA polynucleotide. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by Bergeron. Bergeron teaches amplification and detection of a 16S rRNA polynucleotide (para [0023] - "Highly conserved genes are useful for identification of microorganisms ... the principal targets used for identification purposes are the small subunit (SSU) ribosomal 16S rRNA genes"). As the technical feature was known in the art at the time of the invention, it cannot be considered a special technical feature that would otherwise unify the groups.

Groups I-VII therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.