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(54) **COMPOSITIONS AND METHODS FOR DETECTING STAPHYLOCOCCOUS INFECTIONS**

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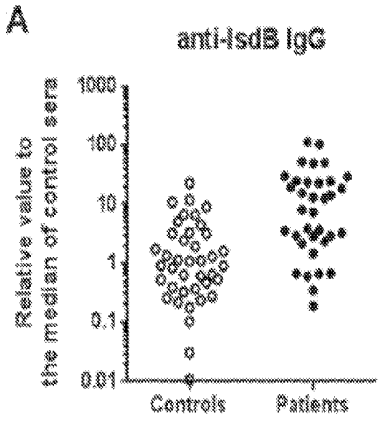
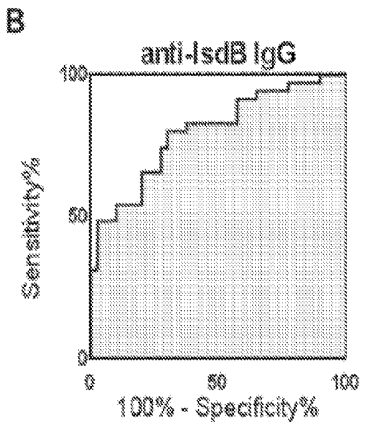
(57) **ABSTRACT**

Provided herein are compositions and methods for detecting and/or diagnosing a *Staphylococcus aureus* (*S. aureus*) infection in a subject. It is a surprising finding of the present invention that an *S. aureus* infection may be detected by using one or more antigens selected from the group consisting of an IsdA antigen, IsdB antigen, CHIPS antigen, and a SCIN antigen. In some embodiments, these *S. aureus* antigens are used to detect antibodies secreted by the plasmablasts from the subject.





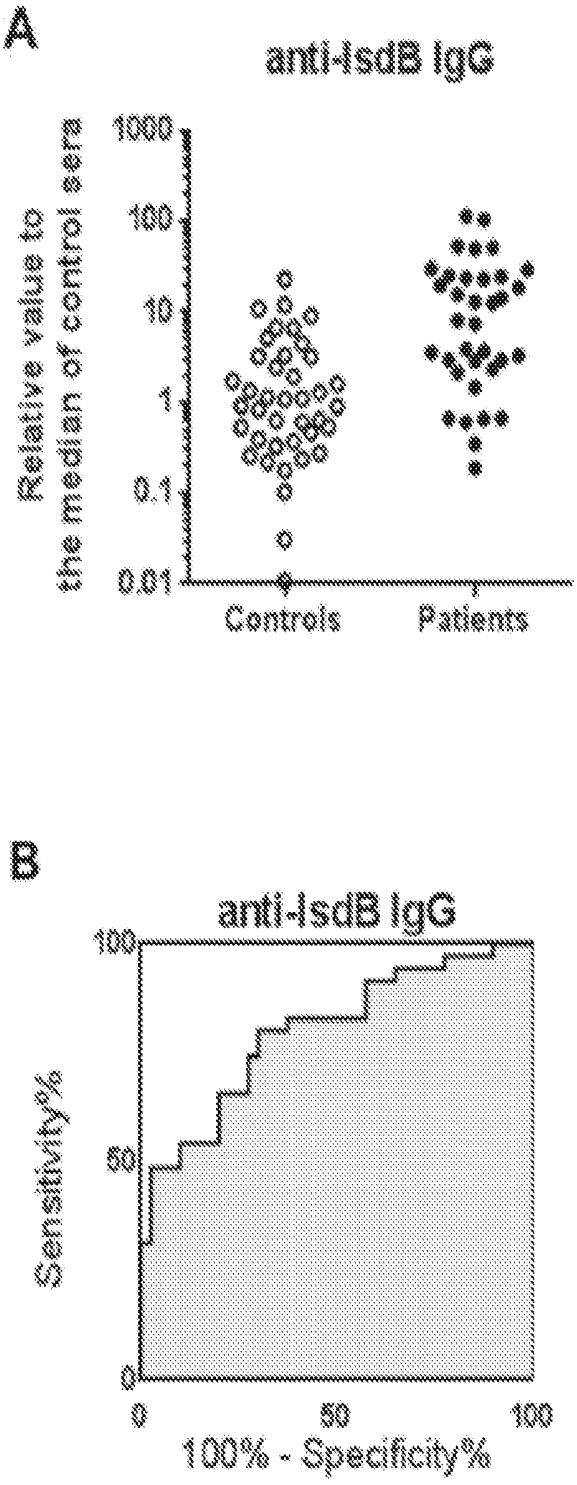

A
anti-IsdB IgG
Relative value to the median of control sera
1000
100
10
1
0.1
0.01
Controls
Patients
B
anti-IsdB IgG
Sensitivity%
100
50
0
0
50
100
100% - Specificity%

FIG 1

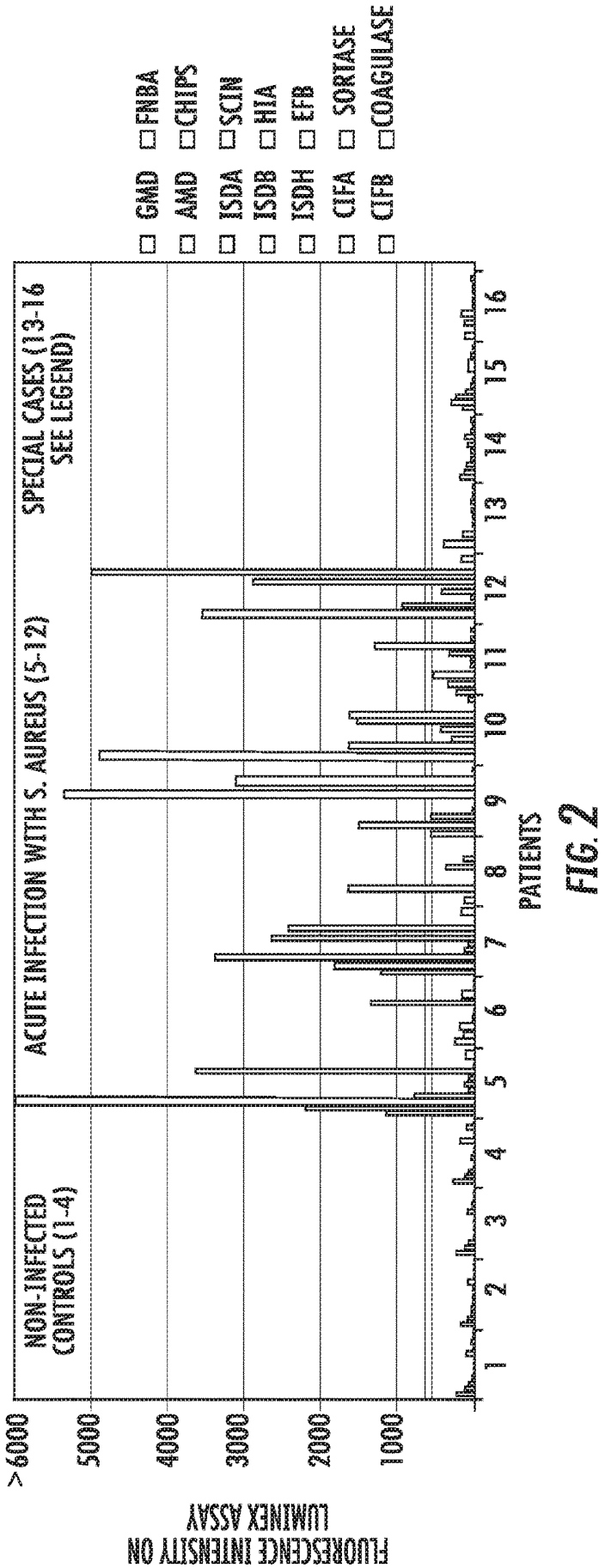

FLUORESCENCE INTENSITY ON LUMINEX ASSAY
>6000
5000
4000
3000
2000
1000
NON-INFECTED CONTROLS (1-4)
ACUTE INFECTION WITH S. AUREUS (5-12)
SPECIAL CASES (13-16 SEE LEGEND)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
PATIENTS
GMD
AMD
ISDA
ISDB
ISDH
CIFA
CIFB
FNBA
CHIPS
SCIN
HIA
EFB
SORTASE
COAGULASE

FIG. 2

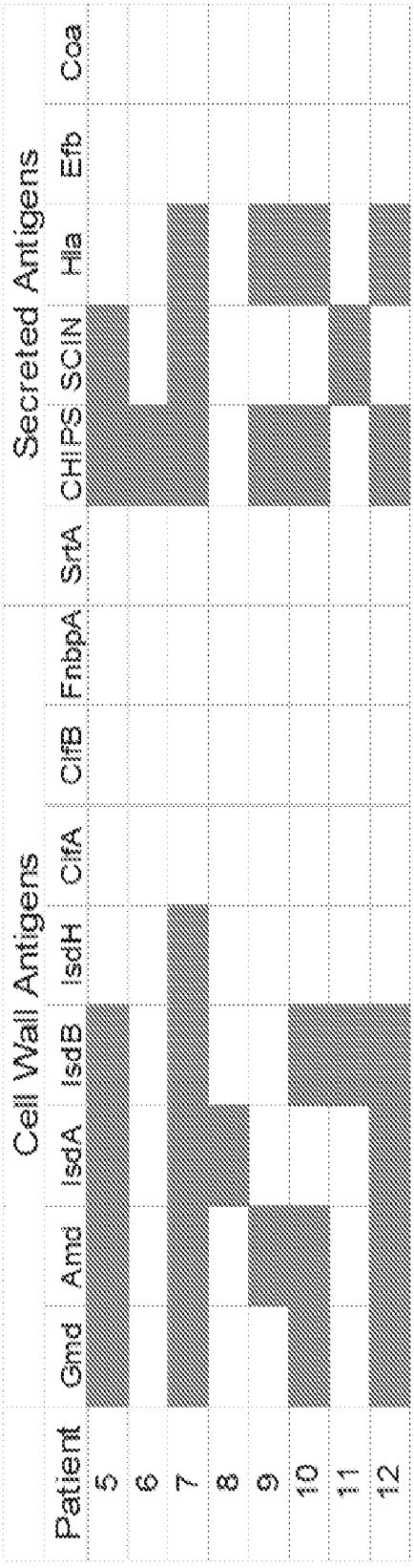

Patient
Cell Wall Antigens
Gmd
Amd
IsdA
IsdB
IsdH
ClfA
ClfB
FnbpA
SrtA
Secreted Antigens
CHIPS
SCIN
Hla
Efb
Coa
5
6
7
8
9
10
11
12

FIG 3

COMPOSITIONS AND METHODS FOR DETECTING STAPHYLOCOCCOUS INFECTIONS

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0001] This invention was made with Government support under Contract No. P30AR061307 awarded by the National Institutes of Health. The U.S. Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

1) Field of the Invention

[0002] The field of the invention is detecting infection in a subject.

2) Description of Related Art

[0003] Infected total joint replacements (TJR) are a significant source of cost and morbidity. Facing years of limited and declining mobility, millions of Americans have had their lives transformed by TJR. In fact, the success of these surgeries has been heralded as the greatest medical achievement of the second half of the Twentieth Century. In 2014, as many as 1.5 million Americans will undergo replacement of a knee or hip and these numbers will grow for the foreseeable future as the population ages and lives longer [Cram P, Lu X, Kates S L, et al., JAMA. 2012 Sep. 26, 308(12):1227-1236; Kurtz S M, Lau E, Ong K, et al., Clin Orthop Relat Res. 2009, October, 467(10):2606-2612]. However, the introduction of a large foreign body into these patients creates an ideal niche for opportunistic pathogens, so it is not surprising that infection is by far the most feared complication of TJR [Berbari E F, Osmon D R, Lahr B, et al., Infect. Control Hosp. Epidemiol. 2012 August, 33(8): 774-781]. Following years of surgical vigilance, the rate of TJR infection has been reduced to 1-2% for total hip and total knee replacements [Ong K L, Kurtz S M, Lau E, et al. J Arthroplasty. 2009 Sep. 24(6 Suppl):105-109; Parvizi J, Azzam K, Ghanem E, et al. Clin Orthop Relat Res. 2009 July, 467(7):1732-1739; Parvizi J, Della Valle C J. AAOS Clinical, J Am Acad Orthop Surg. 2010 December, 18(12): 771-772]. Even with such a modest rate of infection, 15,000 to 30,000 Americans will have their dreams of improved independence and mobility shattered and the healthcare system will absorb billions of dollars in additional costs [Anderson D J, Kaye K S, Chen L F, et al. PLoS One. 2009, 4(12):e8305]. For many patients with infected TJR the outcomes can be severe: between 20 and 40% will end in fusion, amputation or death from infection [Parvizi J, Azzam K, Ghanem E, et al. Clin Orthop Relat Res. 2009 July, 467(7):1732-1739; Cahill J L, Shadbolt B, Scarvell J M, et al. J Orthop Surg (Hong Kong). 2008 April, 16(1):58-65].

[0004] Numerous bacterial species cause TJR infections, but as many as 80% involve Staphylococci in single or mixed infections, and these are about evenly divided between coagulase-positive and coagulase-negative species (Moran E, Byren I, Atkins B L. J Antimicrob Chemother. 2010 November, 65 Suppl 3:iii45-54). Most non-Staphylococci and the coagulase negative Staphylococci respond well to antibiotic therapy. Conversely, in the context of TJR, *S. aureus* (and especially methicillin resistant *S. aureus*; MRSA) is recalcitrant to antibiotics and accounts for the most challenging and costly clinical cases [Parvizi J, Pawasarat I M, Azzam K A, et al. J Arthroplasty. 2010 September, 25(6 Suppl):103-107; Salgado C D, Dash S, Cantey J R, et al. Clin Orthop Relat Res. 2007 August, 461:48-53; Teterycz D, Ferry T, Lew D, et al. Int J Infect Dis. 2010 October, 14(10):e913-918].

[0005] Diagnosis of infected TJR is complicated by several factors [Moran E, Byren I, Atkins B L. J Antimicrob Chemother. 2010 November, 65 Suppl 3:iii45-54. Della Valle C, Parvizi J, Bauer T W, et al. Diagnosis of periprosthetic joint infections of the hip and knee. J Am Acad Orthop Surg. 2010 December; 18(12):760-770]. First, it is uncommon; only one or two patients in one hundred will develop infections. Second, early infections look similar to normal healing with elevated inflammatory markers, redness, pain and swelling at the wound site. For several weeks most infections are not distinguishable from normal healing. Third, the clinical onset is often delayed for months, sometimes years. Fourth, it often presents similarly to other common conditions including seroma, hematoma, deep venous thrombosis or superficial surgical site infection such as cellulitis. Rarely do these infections present in a fulminant manner so providers must wait for the infection to declare itself. Thus, diagnosis typically occurs after the acute phase has passed and a chronic infection is already well established.

[0006] Critically, it is often difficult [DeBonville D. MLO Med Lab Obs. 2012 January, 44(1):26-27] and expensive to obtain a pathogen-containing sample that can be easily tested by culture or PCR and the clinician is compelled to use indirect measures. In fact, the Musculoskeletal Infection Society recently formed a workgroup on diagnostic criteria [Parvizi J, Zmistowski B, Berbari E F, et al. Clin Orthop Relat Res. 2011 November, 469(10:2992-2994]. They proposed the following criteria: sinus tract drainage, two separate positive cultures from the joint, or when four of the following six conditions are met: 1) increased serum erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP); 2) increased synovial leukocyte count; 3) elevated neutrophil percentage; 4) purulence in the joint; 5) isolation of an organism in one culture of joint fluid of tissue; 6) greater than five neutrophils per high-power field of histologic analysis of synovial tissue. While these guidelines are designed to help the physician make accurate treatment decisions, the criteria are non-specific and are limited by the current clinical tests available.

[0007] Early and precise diagnosis of infection is essential. Precise diagnosis obviates the need for empiric broad-spectrum therapies, which have the potential to lead to future pathogen resistance. Furthermore, minimizing the need for empiric broad-spectrum antibiotic therapy with precise diagnosis limits the side effects of unneeded antibiotic therapy such as *Clostridium difficile* infection or renal injury due to antibiotic toxicities [Gonzalez de Molina F J, Ferrer R. Crit Care. 2011, 15(4):175; Yarinsky S, Wheeler W E. W V Med J. 1990 June, 86(6):239-242]. As the pharmaceutical industry continues to make advances in pathogen-specific therapies, the field of diagnostics needs to keep up.

SUMMARY OF THE INVENTION

[0008] Provided herein is a method of determining an *S. aureus* infection in a subject, comprising: 1) generating a plasmablasts-secreted antibody medium by collecting peripheral blood mononuclear cells (PBMCs) from blood in

a subject, removing existing antibodies from the PBMCs and culturing the antibody-free PBMCs in a medium for a period of time to allow secretion of antibodies by plasmablasts contained in the PBMCs into the medium; 2) contacting the plasmablasts-secreted antibody medium with one or more *S. aureus* antigens; 3) detecting the presence of one or more antigen-specific antibodies in the plasmablasts-secreted antibody medium at a level above a control; and 4) determining the *S. aureus* infection in the subject based on the detection of antigen-specific antibodies in step 3). In some embodiments, the one or more *S. aureus* antigens are selected from an IsdA antigen, IsdB antigen, CHIPS antigen, and SCIN antigen. The plasmablasts-secreted antibody medium can be contacted with one, or two, or three, or all of the IsdA antigen, IsdB antigen, CHIPS antigen, and SCIN antigen.

[0009] The plasmablasts-secreted antibody medium can be generated by culturing antibody-free PBMCs for about 1 to about 168 hours. In some embodiments, the antibody-free PBMCs are cultured for about 36 to about 72 hours. In some embodiments, one or more antigen-specific antibodies in the plasmablasts-secreted antibody medium are at a level of at least about 10% more than an antibody control level.

[0010] Also provided herein is a kit for determining if a subject has a *Staphylococcus aureus* (*S. aureus*) infection, wherein the kit includes one or more antigens selected from the group consisting of an IsdA antigen, an IsdB antigen, a CHIPS antigen, and a SCIN antigen; a reservoir for contacting the one or more antigens with the plasmablasts-secreted antibody medium; and one or more compositions for detecting binding between the one or more antigens with one or more antibodies in the plasmablasts-secreted antibody medium. In some embodiments, all of the IsdA antigen, IsdB antigen, CHIPS antigen, and SCIN antigen are comprised within the kit. In some embodiments, the composition for detecting binding is a labelled anti-IgG antibody.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] FIGS. **1**A and **1**B show that Human IgG levels against IsdB are the best single antigen predictor of recent/ongoing infection and they are not clinically useful. A) Dot plot presenting anti-IsdB IgG titers for 40 Controls and 35 patients on a log 10 scale. B) ROC curve presenting the same data. AUC=0.80.

[0012] FIG. **2** shows that the multiplex immunoassay described herein accurately identifies patients with ongoing *S. aureus* infections. FI values for 14 antigens in 16 patients, twelve with suspected *S. aureus* infections and four non-infected controls. Patients 1-4 are non-infected controls; 5-12 were culture-confirmed with *S. aureus;* 13 culture-confirmed with *P. aeruginosa;* 14 and 15, >30 days antibiotic therapy; 16, culture negative. Red line=positive-negative cut-off=twice highest FI value measured for any antigen in a control sample.

[0013] FIG. **3** shows the *S. aureus* antigens that were detected by IgG in the MENSA of infected patients. FI values that were at least twice the maximum FI value for any antigen among the non-infected controls are presented as filled-in red boxes.

DETAILED DESCRIPTION OF THE INVENTION

[0014] Provided herein are compositions and methods for detecting and/or diagnosing a *Staphylococcus aureus* (*S. aureus*) infection in a subject. It is a surprising finding of the present invention that an *S. aureus* infection may be detected by using one or more antigens selected from the group consisting of an IsdA antigen, IsdB antigen, CHIPS antigen, and a SCIN antigen. In some embodiments, these *S. aureus* antigens are used to detect antibodies secreted by the plasmablasts from the subject.

Definitions

[0015] Terms used throughout this application are to be construed with ordinary and typical meaning to those of ordinary skill in the art. However, Applicants desire that the following terms be given the particular definition as defined below.

[0016] As used in the specification and claims, the singular form "a," "an," and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof.

[0017] The terms "about" and "approximately" are defined as being "close to" as understood by one of ordinary skill in the art. In one non-limiting embodiment the terms are defined to be within 10%. In another non-limiting embodiment, the terms are defined to be within 5%. In still another non-limiting embodiment, the terms are defined to be within 1%.

[0018] The term "antibody" is used in the broadest sense, and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, and multispecific antibodies (e.g., bispecific antibodies). Antibodies (Abs) and immunoglobulins (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific target, immunoglobulins include both antibodies and other antibody-like molecules which lack target specificity. Native antibodies and immunoglobulins are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end.

[0019] The term "antibody fragment" refers to a portion of a full-length antibody, generally the target binding or variable region. Examples of antibody fragments include Fab, Fab', $F(ab')_2$ and Fv fragments. The phrase "functional fragment or analog" of an antibody is a compound having qualitative biological activity in common with a full-length antibody. For example, a functional fragment or analog of an anti-IgE antibody is one which can bind to an IgE immunoglobulin in such a manner so as to prevent or substantially reduce the ability of such molecule from having the ability to bind to the high affinity receptor, FcεRI. As used herein, "functional fragment" with respect to antibodies, refers to Fv, F(ab) and $F(ab')_2$ fragments. An "Fv" fragment is the minimum antibody fragment which contains a complete target recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in a tight, non-covalent association (V_H-V_L dimer). It is in this configuration that the three CDRs of each variable domain interact to define an target binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer target binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three

CDRs specific for a target) has the ability to recognize and bind target, although at a lower affinity than the entire binding site. "Single-chain Fv" or "sFv" antibody fragments comprise the V_H and V_L domains of an antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for target binding.

[0020] The Fab fragment contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. F(ab') fragments are produced by cleavage of the disulfide bond at the hinge cysteines of the $F(ab')_2$ pepsin digestion product. Additional chemical couplings of antibody fragments are known to those of ordinary skill in the art.

[0021] The term "B cell" includes B-1 cells, B-2 cells, plasma B cells, memory B cells, and plasmablasts. The term "peripheral blood B cell" refers to a B cell present in the peripheral blood of an individual. Peripheral blood B cells include, but are not limited to, immature/transitional B cells, naïve B cells, memory B cells, and plasmablasts.

[0022] The terms "cell," "cell line" and "cell culture" include progeny. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Variant progeny that have the same function or biological property, as screened for in the originally transformed cell, are included.

[0023] A "composition" is intended to include a compound, an active agent, or a combination of active agent with another agent or compound or composition, inert (for example, a detectable agent or label) or active, such as an adjuvant.

[0024] As used herein, the term "comprising" is intended to mean that the compositions and methods include the recited elements, but not excluding others. "Consisting essentially of" when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives, and the like. "Consisting of" shall mean excluding more than trace elements of other ingredients and substantial method steps for administering the compositions of this invention. Embodiments defined by each of these transition terms are within the scope of this invention.

[0025] A "control" is an alternative subject or sample used in an experiment for comparison purpose. A control can be "positive" or "negative."

[0026] As used herein, the term "culture supernatant" refers to a medium in which a cell or cells have developed or proliferated. In some embodiments, the culture supernatant is a plasmablasts-secreted antibody medium obtained following approximately 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 24 hours, 36 hours, 48 hours, 72 hours, 84 hours, 108 hours, 132 hours, 156 hours, or 168 hours of cell development or proliferation in the medium.

[0027] The word "label" when used herein refers to a detectable compound or composition which can be conjugated directly or indirectly to a molecule or protein, e.g., an antibody. The label may itself be detectable (e.g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

[0028] The term "lymphocyte" refers to natural killer cells, T cells, or B cells.

[0029] "Mammal" for purposes of treatment refers to any animal classified as a mammal, including human, domestic and farm animals, nonhuman primates, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc.

[0030] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single target site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the target. In addition to their specificity, monoclonal antibodies are advantageous in that they may be synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies for use with the present invention may be isolated from phage antibody libraries using the well-known techniques. The parent monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler and Milstein, Nature 256, 495 (1975), or may be made by recombinant methods.

[0031] The terms "peripheral blood mononuclear cell" and "PBMC" are used interchangeably herein and refer to as any blood cell with a round nucleus (i.e. a lymphocyte, a monocyte, or a macrophage). As used herein, the term "PBMC culture" refers to any PBMC obtained from a subject's blood that grow or develop in a medium in vitro. A "PBMC culture supernatant" refers herein to a medium in which the existing antibodies in the PBMC-containing blood is removed and obtained from a subject's blood have developed or proliferated.

[0032] "Plasmablasts" are defined herein as white blood cells that secrete large volumes of antibodies. They are antibody-secreting B cells that may be identified by flow cytometry as $CD19^{low}CD20^{-}CD27^{high}CD38^{high}CD138^{\pm}$. In some embodiments, plasmablasts appear in the blood shortly after infection and persist as long as antigen is detectable. In some embodiments, plasmablasts have differentiated from memory B cells.

[0033] The term "polypeptide" is used in its broadest sense to refer to a compound of two or more subunit amino acids, amino acid analogs, or peptidomimetics. The subunits may be linked by peptide bonds. In another embodiment, the subunit may be linked by other bonds, e.g. ester, ether, etc. As used herein the term "amino acid" refers to either natural and/or unnatural or synthetic amino acids, including glycine and both the D or L optical isomers, and amino acid analogs and peptidomimetics. A peptide of three or more amino acids is commonly called an oligopeptide if the peptide chain is

short. If the peptide chain is long, the peptide is commonly called a polypeptide or a protein.

[0034] As used herein, the term "solid support" means a non-aqueous matrix to which the one or more *S. aureus* antigens of the present invention can adhere and is not limited to a specific type of support. Rather a large number of supports are available and are known to one of ordinary skill in the art. Solid phase supports include silica gels, resins, derivatized plastic films, glass beads, cotton, plastic beads, and alumina gels. As used herein, "solid support" also includes synthetic antigen-presenting matrices, cells, and liposomes. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (e.g. an affinity chromatography column). A suitable solid support may be selected on the basis of desired end use and suitability for various protocols.

[0035] The term "subject" is defined herein to include animals such as mammals, including, but not limited to, primates (e.g., humans), cows, sheep, goats, horses, dogs, cats, rabbits, rats, mice and the like. In some embodiments, the subject is a human.

[0036] The term "variable" in the context of variable domain of antibodies, refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular target. However, the variability is not evenly distributed through the variable domains of antibodies. It is concentrated in three segments called complementarity determining regions (CDRs) also known as hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely a adopting a .beta.-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the .beta.-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the target binding site of antibodies (see Kabat et al.) As used herein, numbering of immunoglobulin amino acid residues is done according to the immunoglobulin amino acid residue numbering system of Kabat et al., (Sequences of Proteins of Immunological Interest, National Institute of Health, Bethesda, Md. 1987), unless otherwise indicated.

DETAILED DESCRIPTION

[0037] As indicated above, provided herein are compositions and methods for detecting and/or diagnosing a *Staphylococcus aureus* (*S. aureus*) infection in a subject. It is a surprising finding of the present invention that an *S. aureus* infection may be detected by using an *S. aureus* antigen and a peripheral blood mononuclear cell (PBMC) culture supernatant wherein the PBMC is obtained from the subject. In some embodiments, one or more *S. aureus* antigens is selected from the group consisting of an IsdA antigen, an IsdB antigen, a CHIPS antigen, and a SCIN antigen.

[0038] Provided herein is a method of determining *S. aureus* infection in a subject, comprising: 1) generating a plasmablasts-secreted antibody medium by collecting peripheral blood mononuclear cells (PBMCs) from blood in a subject, removing existing antibodies from the PBMCs and culturing the antibody-free PBMCs in a medium for a period of time to allow secretion of antibodies by plasmablasts contained in the PBMCs into the medium; 2) contacting the plasmablasts-secreted antibody medium with one or more *S. aureus* antigens; 3) detecting the presence of one or more antigen-specific antibodies in the plasmablasts-secreted antibody medium at a level above a control; and 4) determining the *S. aureus* infection in the subject based on the detection of antigen-specific antibodies in step 3).

[0039] In some embodiments, the PBMCs are isolated from a subject's blood sample via a Ficoll-Paque method. Ficoll-Paque methods of PBMC isolation are well known to those of ordinary skill in the art, and all such methods are included herein. In some embodiments, the Ficoll-Pacque method includes the use of POLYMORPHPREP™. In some embodiments, the antibody-free PBMCs are cultured in a medium at approximately 37° C. for approximately 12 hours, 24 hours, 36 hours, 48 hours, 72 hours, or 84 hours. In some embodiments, the medium is a DMEM, RPMI 1640, and SFM_4MAB (Hyclone Laboratories, Serum Free Medium for Monoclonal Antibodies). In some embodiments, the medium is supplemented with a fetal bovine serum. The fetal bovine serum may be at about 5% v/v, about 10% v/v, about 15% v/v, about 20% v/v, or about 25% v/v. Antibiotics such as gentamicin may also be added to the medium. In some embodiments, the PBMCs are cultured in the presence of about 5% CO_2.

[0040] In some embodiments, the plasmablasts is optionally further removed from the plasmablasts-secreted antibody medium to generate a substantially cell-free medium.

[0041] As used herein, "a control level" refers to a level of antigen-antibody binding in the plasmablasts-secreted antibody medium from a healthy, non-infection subject or a subject with an infection that has been cleaned. As used herein, the term "at a level above a control level" refers to a level of antigen-antibody binding that is at least about 10% more, about 15% more, about 20% more, about 30% more, about 40% more, about 50% more, about 60% more, about 70% more, about 80% more, about 90% more, or about 100% more than the control level. In one embodiment, the term "at a level above a control level" means a level of greater than 10%, 20%, 30%, 40%, 50%, 60% 70%, 80%, 90%, 100%, or 150% or 200% more than the control level.

[0042] In some embodiments, the *S. aureus* antigens are selected from the group consisting of an IsdA antigen, an IsdB antigen, a CHIPS antigen, and a SCIN antigen. In some embodiments, the plasmablasts-secreted antibody medium is contacted with two or three or all of an IsdA antigen, an IsdB antigen, a CHIPS antigen, and a SCIN antigen.

[0043] The term "IsdA" refers to an *S. aureus* surface or cell wall expressed polypeptide also referred to as iron-regulated surface determinant protein A. In some embodiments, the IsdA antigen comprises the sequence of SEQ ID NO:1, or a polypeptide sequence having at or greater than about 80%, about 85%, about 90%, about 95%, or about 98% homology with SEQ ID NO:1, or a polypeptide comprising a portion of SEQ ID NO:1. In some embodiments, the IsdA antigen comprises additional polypeptide sequences that allow for purification of the antigen and/or attachment of the antigen to a solid support. In some embodiments, the IsdA antigen comprises a polypeptide sequence of SEQ ID NO:1 and an N-terminal six histidine purification sequence of MHHHHHH (SEQ ID NO:5). In other embodiments, the IsdA antigen comprises a polypeptide sequence of SEQ ID NO:1 and a C-terminal bioti-

nylation sequence of LNDIFEAQKIEWHG (SEQ ID NO:6). In some embodiments, the IsdA antigen comprises SEQ ID NO:1, SEQ ID NO: 5 and SEQ ID NO:6.

[0044] The term "IsdB" refers to an *S. aureus* surface or cell wall expressed polypeptide also referred to as iron-regulated surface determinant protein B. In some embodiments, the IsdB antigen comprises the sequence of SEQ ID NO:2, or a polypeptide sequence having at or greater than about 80%, about 85%, about 90%, about 95%, or about 98% homology with SEQ ID NO:2, or a polypeptide comprising a portion of SEQ ID NO:2. In some embodiments, the IsdB antigen comprises additional polypeptide sequences that allow for purification of the antigen and/or attachment of the antigen to a solid support. In some embodiments, the IsdB antigen comprises a polypeptide sequence of SEQ ID NO:2 and an N-terminal six histidine purification sequence of MHHHHHH (SEQ ID NO:5). In other embodiments, the IsdB antigen comprises a polypeptide sequence of SEQ ID NO:2 and a C-terminal biotinylation sequence of LNDIFEAQKIEWHG (SEQ ID NO:6). In some embodiments, the IsdB antigen comprises SEQ ID NO:2, SEQ ID NO:5 and SEQ ID NO:6.

[0045] The term "CHIPS" refers to an *S. aureus* exoprotein also referred to as chemotaxis inhibitory protein. In some embodiments, the CHIPS antigen comprises the sequence of SEQ ID NO:3, or a polypeptide sequence having at or greater than about 80%, about 85%, about 90%, about 95%, or about 98% homology with SEQ ID NO:3, or a polypeptide comprising a portion of SEQ ID NO:3. In some embodiments, the CHIPS antigen comprises additional polypeptide sequences that allow for purification of the antigen and/or attachment of the antigen to a solid support. In some embodiments, the CHIPS antigen comprises a polypeptide sequence of SEQ ID NO:3 and an N-terminal six histidine purification sequence of MHHHHHH (SEQ ID NO:5). In other or further embodiments, the CHIPS antigen comprises a polypeptide sequence of SEQ ID NO:3 and a C-terminal biotinylation sequence of LNDIFEAQKIEWHG (SEQ ID NO:6). In some embodiments, the CHIPS antigen comprises SEQ ID NO:3, SEQ ID NO: 5 and SEQ ID NO:6.

[0046] The term "SCIN" refers to an *S. aureus* exoprotein also referred to as staphylococcal complement inhibitor. In some embodiments, the SCIN antigen comprises the sequence of SEQ ID NO:4, or a polypeptide sequence having at or greater than about 80%, about 85%, about 90%, about 95%, or about 98% homology with SEQ ID NO:4, or a polypeptide comprising a portion of SEQ ID NO:4. In some embodiments, the SCIN antigen comprises additional polypeptide sequences that allow for purification of the antigen and/or attachment of the antigen to a solid support. In some embodiments, the SCIN antigen comprises a polypeptide sequence of SEQ ID NO:4 and an N-terminal six histidine purification sequence of MHHHHHH (SEQ ID NO:5). In other or further embodiments, the SCIN antigen comprises a polypeptide sequence of SEQ ID NO:4 and a C-terminal biotinylation sequence of LNDIFEAQKIEWHG (SEQ ID NO:6). In some embodiments, the SCIN antigen comprises SEQ ID NO:4, SEQ ID NO: 5 and SEQ ID NO:6.

[0047] As indicated above, provided herein is a method of determining if a subject has a *S. aureus* infection, comprising: 1) generating a plasmablasts-secreted antibody medium by collecting peripheral blood mononuclear cells (PBMCs) from blood in a subject, removing existing antibodies from the PBMCs and culturing the antibody-free PBMCs in a medium for a period of time to allow secretion of antibodies by plasmablasts contained in the PBMCs into the medium; 2) contacting the plasmablasts-secreted antibody medium with one or more *S. aureus* antigens selected from the group consisting of an IsdA antigen, an IsdB antigen, a CHIPS antigen, and a SCIN antigen; 3) detecting the presence of one or more antigen-specific antibodies in the plasmablasts-secreted antibody medium at a level above a control; and 4) determining the *S. aureus* infection in the subject based on the detection of antigen-specific antibodies in step 3). In some embodiments, the binding of antigen-specific antibodies to one or two of the IsdA antigen, IsdB antigen, CHIPS antigen, and SCIN antigen indicates the subject has a *S. aureus* infection. In some embodiments, the binding of antigen-specific antibodies to three of the IsdA antigen, IsdB antigen, CHIPS antigen, and SCIN antigen indicates the subject has a *S. aureus* infection. In some embodiments, the binding of all of the IsdA antigen, IsdB antigen, CHIPS antigen, and SCIN antigen indicates the subject has a *S. aureus* infection.

[0048] Binding of antigen to an antibody may be detected using any method known to those of ordinary skill in the art. Binding of antigen to antibody may be detected using an immunoassay. Many types and formats of immunoassays are known and all are suitable for detecting the disclosed biomarkers. Examples of immunoassays are enzyme linked immunosorbent assays (ELISAs), enzyme linked immunospot assay (ELISPOT), radioimmunoassays (RIA), radioimmune precipitation assays (RIPA), immunobead capture such as assays involving distinctly colored antigen bound Lumavidin beads and phycoerythrin (PE) labelled anti-Ig, Western blotting, dot blotting, gel-shift assays, Flow cytometry, protein arrays, multiplexed bead arrays, magnetic capture, in vivo imaging, fluorescence resonance energy transfer (FRET), and fluorescence recovery/localization after photobleaching (FRAP/FLAP).

[0049] In general, immunoassays involve contacting a sample suspected of containing a molecule of interest (such as an *S. aureus* antigen) with an antibody to the molecule of interest or contacting an antibody to a molecule of interest (such as antibodies to an *S. aureus* antigen) with a molecule that can be bound by the antibody, as the case may be, under conditions effective to allow the formation of immunocomplexes. Contacting a sample with the antibody to the molecule of interest or with the molecule that can be bound by an antibody to the molecule of interest under conditions effective and for a period of time sufficient to allow the formation of immune complexes (primary immune complexes) is generally a matter of simply bringing into contact the molecule or antibody and the sample and incubating the mixture for a period of time long enough for the antibodies to form immune complexes with, i.e., to bind to, any molecules (e.g., antigens) present to which the antibodies can bind. In many forms of immunoassay, the sample-antibody composition, such as a tissue section, ELISA plate, dot blot or Western blot, can then be washed to remove any non-specifically bound antibody species, allowing only those antibodies specifically bound within the primary immune complexes to be detected.

[0050] Immunoassays can include methods for detecting or quantifying the amount of a molecule of interest (such as antibodies to an *S. aureus* antigen) in a sample, which methods generally involve the detection or quantitation of any immune complexes formed during the binding process.

In general, the detection of immunocomplex formation is well known in the art and can be achieved through the application of numerous approaches. These methods are generally based upon the detection of a label or marker, such as any radioactive, fluorescent, biological or enzymatic tags or any other known label.

[0051] As used herein, a label can include a fluorescent dye, a member of a binding pair, such as biotin/streptavidin, a metal (e.g., gold), or an epitope tag that can specifically interact with a molecule that can be detected, such as by producing a colored substrate or fluorescence. Substances suitable for detectably labeling proteins include fluorescent dyes (also known herein as fluorochromes and fluorophores) and enzymes that react with colorimetric substrates (e.g., horseradish peroxidase). Furthermore, in the case where multiple antigens are reacted with a single array, each antigen can be labeled with a distinct fluorescent compound for simultaneous detection. Labeled spots on the array are detected using a fluorimeter, the presence of a signal indicating an antigen bound to a specific antibody.

[0052] Also provided herein is a kit for determining if a subject has a *Staphylococcus aureus* (*S. aureus*) infection, comprising one or more antigens selected from the group consisting of an IsdA antigen, an IsdB antigen, a CHIPS antigen, and a SCIN antigen; a reservoir for contacting the one or more antigens with a plasmablasts-secreted antibody medium derived from the subject; and a composition for detecting binding between the one or more antigens with one or more antibodies in the plasmablasts-secreted antibody medium. In some embodiments, one or two of the IsdA antigen, IsdB antigen, CHIPS antigen, and SCIN antigen are comprised within the kit. In other embodiments, three of the IsdA antigen, IsdB antigen, CHIPS antigen, and SCIN antigen are comprised within the kit. In other embodiments, all four of the IsdA antigen, IsdB antigen, CHIPS antigen, and SCIN antigen are comprised within the kit. The one or more antigens can be bound to a solid support in or on the reservoir in any manner. In some embodiments, the one or more antigens are bound to the reservoir itself in an array.

[0053] The one or more compositions for detecting binding between the one or more antigens with one or more antibodies in the plasmablasts-secreted antibody medium can be any known to one of ordinary skill in the art. In some embodiments, the composition for detecting binding is a labelled anti-IgG antibody. As indicated above, the word "label" when used herein includes a detectable compound or composition which can be conjugated directly or indirectly to a molecule or protein, e.g., an antibody. The label may itself be detectable (e.g., radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable. In some embodiments, the label is fluorescent.

[0054] It should also be understood that the foregoing relates to preferred embodiments of the present invention and that numerous changes may be made therein without departing from the scope of the invention. The invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof, which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims. All patents, patent applications, and publications referenced herein are incorporated by reference in their entirety for all purposes.

EXAMPLES

Example 1

Identification of Diagnostic *S. aureus* Antigens

[0055] Antigen Selection.

[0056] Fourteen antigens were selected for analysis. Ten are cell-wall-associated enzymes or binding proteins and four are secreted factors that interfere with the human immune response. Additional selection criteria included: 1) conservation among *S. aureus* strains; 2) single subunit; 3) a role in one or more essential functions; 4) accessibility from outside the cell wall; and 5) evidence that antibody titer increased during infection. The following were several antigens that were chosen: cell wall modifying enzymes glucosaminidase (Gmd), amidase (Amd) [Gedbjerg N, LaRosa R, Hunter J G, et al. J Bone Joint Surg Am. 2013 Nov. 20; 95(22):e171; Varrone J, Dussmann E, Yi Y, et al. Orthopaedic Research Society 20122012; Varrone J, Li D, Daiss J, et al. IBMS BoneKEy 2011; 8(4):187-194; Varrone J J, Bentley K L D, Bello-Irizarry S N, et al. Journal of Orthopaedic Research. 2014; in press; Bose J L, Lehman M K, Fey P D, et al. PLoS One. 2012; 7(7):e42244; Brady R A, O'May G A, Leid J G, et al. Infect Immun. 2011 April; 79(4):1797-1803; Biswas R, Voggu L, Simon U K, et al. FEMS Microbiol Lett. 2006 June; 259(2):260-268; Gotz F, Heilmann C, Stehle T. Int J Med Microbiol. 2013 Dec. 1; Zoll S, Patzold B, Schlag M, et al. PLoS Pathog. 2010 March; 6(3):e1000807; Zoll S, Schlag M, Shkumatov A V, et al. J Bacteriol. 2012 August; 194(15):3789-3802; Buttner F M, Zoll S, Nega M, et al. J Biol Chem. 2014 Apr. 18; 289(16): 11083-11094; Gotz F, Heilmann C, Stehle T. Int J Med Microbiol. 2014 March; 304(2):156-163], and sortase (SrtA) [Kim H K, Kim H Y, Schneewind O, et al. FASEB J. 2011 October; 25(10):3605-3612; Chen F, Liu B, Wang D, et al. FEMS Microbiol Lett. 2014 February; 351(1):95-103; Maresso A W, Schneewind O. Pharmacol Rev. 2008 March; 60(1):128-141], the iron-sequestering proteins IsdA [Clarke S R, Brummell K J, Horsburgh M J, et al. J Infect Dis. 2006 Apr. 15; 193(8):1098-1108; Clarke S R, Foster S J. Infect Immun. 2008 April; 76(4):1518-1526; Kim H K, DeDent A, Cheng A G, et al. Vaccine. 2010 Aug. 31; 28(38):6382-6392], IsdB [Kim H K, DeDent A, Cheng A G, et al. Vaccine. 2010 Aug. 31; 28(38):6382-6392; Torres V J, Pishchany G, Humayun M, et al. J Bacteriol. 2006 December; 188(24): 8421-8429; Zapotoczna M, Jevnikar Z, Miajlovic H, et al. Cell Microbiol. 2013 June; 15(6):1026-1041] and IsdH [Foster T J, Geoghegan J A, Ganesh V K, et al. Nat Rev Microbiol. 2014 January; 12(1):49-62; Visai L, Yanagisawa N, Josefsson E, et al. Microbiology. 2009 March; 155(Pt 3):667-679], and the adhesins ClfA [Ganesh V K, Rivera J J, Smeds E, et al. PLoS Pathog. 2008 November; 4(11): e1000226; Li Y, Li Z, Li Y, et al. Wei Sheng Wu Xue Bao. 2013 Sep. 4; 53(9):966-975; Li Y, Liu Y H, Li Z J, et al. Res Vet Sci. 2013 June; 94(3):490-495; Arrecubieta C, Matsunaga I, Asai T, et al. J Infect Dis. 2008 Aug. 15; 198(4): 571-575; Patti J M, Allen B L, McGavin M J, et al. Annu Rev Microbiol. 1994; 48:585-617], ClfB [Corrigan R M, Miajlovic H, Foster T J. BMC Microbiol. 2009; 9:22; Ni

Eidhin D, Perkins S, Francois P, et al. Mol Microbiol. 1998 October; 30(2):245-257], FnbpA [Bingham R J, Rudino-Pinera E, Meenan N A, et al. Proc Natl Acad Sci USA. 2008 Aug. 26; 105(34):12254-12258; Edwards A M, Potts J R, Josefsson E, et al. PLoS Pathog. 2010; 6(6):e1000964; Stemberk V, Jones R P, Moroz O, et al. J Biol Chem. 2014 May 2; 289(18):12842-12851], and coagulase (Coa) [Cheng A G, DeDent A C, Schneewind O, et al. A play in four acts: *Trends Microbiol.* 2011 May; 19(5):225-232; Cheng A G, McAdow M, Kim H K, et al. *PLoS Pathog.* 2010; 6(8): e1001036].

[0057] The selected secreted antigens were alpha hemolysin (Hla) [Chua K Y, Monk I R, Lin Y H, et al. BMC Microbiol. 2014; 14:31; Bubeck Wardenburg J, Schneewind O. J Exp Med. 2008 Feb. 18; 205(2):287-294], CHIPS [Bunschoten A, Feitsma L J, Kruijtzer J A, et al. Bioorg Med Chem Lett. 2010 Jun. 1; 20(11):3338-3340; Bunschoten A, Ippel J H, Kruijtzer J A, et al. Amino Acids. 2011 February; 40(2):731-740; Postma B, Poppelier M J, van Galen J C, et al. J Immunol. 2004 Jun. 1; 172(11):6994-7001; Powers M E, Bubeck Wardenburg J. PLoS Pathog. 2014 February; 10(2):e1003871; van Wamel W J, Rooijakkers S H, Ruyken M, et al J Bacteriol. 2006 February; 188(4):1310-1315], SCIN [Jongerius I, Kohl J, Pandey M K, et al. J Exp Med. 2007 Oct. 1; 204(10):2461-2471; Jongerius I, Puister M, Wu J, et al. J Immunol. 2010 January 1; 184(1):420-425; Rooijakkers S H, Milder F J, Bardoel B W, et al. J Immunol. 2007 Sep. 1; 179(5):2989-2998; Rooijakkers S H, van Strijp J A. Mol Immunol. 2007 January; 44(1-3):23-32] and Efb [Ko Y P, Liang X, Smith C W, et al. J Biol Chem. 2011 Mar. 18; 286(11):9865-9874; Ricklin D, Ricklin-Lichtsteiner S K, Markiewski M M, et al. *J Immunol.* 2008 Dec. 1; 181(11): 7463-7467] each of which interferes with human immune function and the signature enzyme of *S. aureus*.

[0058] Antigen Expression.

[0059] For each antigen the process was the same: i) a consensus sequence was created using Geneious software on *S. aureus* sequences compiled from GenBank; ii) sequences known or likely to be present on other *S. aureus* proteins were removed; iii) sequences encoding domains likely to cause problems during expression in *E. coli* such as those that span membranes were removed; iv) sequences encoding an N-terminal hexahistidine to facilitate purification and a C-terminal 14-amino acid AviTag biotinylation site to facilitate immobilization on Lumavidin beads were added; v) the chimeric, truncated gene was synthesized de novo using codon optimization for *E. coli*; vi) the synthetic gene was expressed in strains of *E. coli* co-expressing the biotinylating enzyme BirA [Kay B K, Thai S, Volgina V V. Methods Mol Biol. 2009; 498:185-196]; and vii) the recombinant protein was harvested and purified by metal chelation chromatography.

[0060] Human Serum Samples.

[0061] Serum samples were collected from 75 patients under a protocol approved by a Research Subjects Review Board. Forty were from non-infected control patients; 35 were from cases with culture-confirmed, deep-seated musculoskeletal infections with *S. aureus*. Sera were stored at −80° C. until use.

[0062] Whole blood was collected by a phlebotomist into two 10 mL green-topped sodium heparin tubes, mixed by inversion to distribute the anticoagulant, and kept between 20 and 30° C. during transit to the laboratory within four hours. 20 mL of commercially available density reagent, Polymorphprep, was added to a 50 mL conical, screw-capped tube. Then, 10-20 mL of a single patient's anti-coagulated whole blood was layered on top of the density reagent and the capped tubes were centrifuged at 500×g in a swinging bucket centrifuge kept at 25° C. for 35 minutes. At the end of the centrifugation, red blood cells were at the bottom of the tube, neutrophils were present as a band just above the red blood cells and peripheral blood mononuclear cells (PBMC), which PBMC include the circulating plasmablasts, formed a layer at the boundary of the density reagent and the plasma. The PBMC were then carefully collected by aspiration from the plasma:density agent boundary and washed four times in DMEM always keeping the temperature between 20° C. and 37° C. The washed cells were then placed in 10 mL of DMEM supplemented with fetal bovine serum (20% v/v) and gentamicin (50 μg/ml) and cultured at 37° C. for 24-72 hours in a standard cell culture incubators with 5% CO_2. Following the cell culture, the culture medium (MENSA) was harvested, centrifuged at 800×g for 5 minutes and frozen in aliquots for analysis by the multiplex immunoassay described above.

[0063] Multiplex Immunoassay.

[0064] Each purified *S. aureus* antigen was then immobilized on a distinctly colored preparation of Lumavidin beads to yield a preparation of beads that displays only one antigen and possesses a unique color signature in flow cytometry. The antigen-laden Lumavidin beads were then combined and incubated with MENSA, washed, incubated with a secondary reagent: phycoerythrin(PE)-conjugated goat anti-human IgG (or IgM), and then analyzed on a Bio-Plex 200 (BioRad). The Fluorescence Intensity (FI) of PE associated with each color bead was measured as the antigen specific signal. The mean of 50-100 beads of each specificity is reported herein. Titers are expressed as multiples of the median of the control population; positive control is 10,000-fold diluted pool of sera reactive with all 14 antigens.

[0065] Antibodies in Serum are Poor Predictors of Infection.

[0066] Representative data from the best single antigen predictor of recent or ongoing infection, the iron-sequestering cell wall protein IsdB, is presented in FIG. **1**. Several features are immediately apparent. First, the control population has measurable levels of anti-IsdB and the range of titers exceeds two orders of magnitude. Second, the infected patients have a similarly wide distribution of values that is about a half an order of magnitude higher. Thus there is a high, noisy background level in the non-infected controls and a small, noisy elevation in titers of the infected patients. The dot plot in FIG. **1**A presents this clearly and the derived ROC curve in FIG. **1**B illustrates that the clinical predictive power is modest (Area-under-the-curve (AUC)=0.80)). Combinations of antibody titers improve clinical prediction slightly: multivariate analysis of titers for all 14 antigens yield an AUC just under 0.90. Titers for IgM had no predictive power, AUC 0.5-0.55. (data not shown).

[0067] Initial Data Using Circulating Plasmablasts is Encouraging: 100% Sensitivity, 100% Specificity in a Small Cohort.

[0068] The potential utility, simplicity and economy of using the host's immune response as a clinical predictor of ongoing infection is lost in the jaws of high, noisy background and low, noisy signal. The hypothesis set forth herein is that one can break through this constraint by measuring the antibody secreted in vitro by circulating plasmablasts

which are present only during active infection. To test this idea, whole blood was collected from patients with culture-confirmed prosthetic joint infections and from patients presenting in the Emergency Department who were subsequently admitted to Orthopaedics with suspected implant-associated infections. The peripheral blood mononuclear cells (PBMCs) which contain circulating plasmablasts were harvested and placed in culture for 72 hours as described above. Then the culture medium enriched for newly synthesized antibody (MENSA) was obtained and the abundance of IgG for each of the 14 antigens was measured in the multiplex immunoassay described above.

[0069] The results from the first sixteen patients are presented in FIG. **2**. The Fluorescence Intensity (FI) values obtained from undiluted MENSA for each of the 14 antigens are presented as colored vertical bars. Patients numbered 1-4 were non-infected controls and one can see that they have very little IgG against any of the antigens. The origin of the tiny signals observed is under investigation and may be completely eliminated. Patients numbered 5-12 each had ongoing culture-confirmed infections and each had MENSA bearing IgG for one or more of the 14 antigens. Patients numbered 13-16 illustrate other conditions that should be negative. Patient number 13 had a culture-confirmed *P. aeruginosa* infection; Patient number 16 remained culture negative for any pathogen. Patients numbered 14 and 15 had culture-confirmed *S. aureus* infections several months before and had been on extensive antibiotic therapy for at least 30 days prior to our sampling, a promising result suggesting the ASC decline when therapy is successful.

[0070] As shown in FIG. **3**, only three antigens were required to identify all eight of the infected patients (CHIPS, IsdA and (IsdB or SCIN)). The response to each antigen in FIG. **2** was considered positive if it exceeded the highest value for any antigen among the controls by at least a factor of two (shown as red squares in FIG. **3**) and negative if it did not (shown as white squares in FIG. **3**).

SEQUENCE LISTING

SEQ ID NO:1
GATEATNATNNQSTQVSQATSQPINFQVQKDGSSEKSHMDDYMQHPGKVI
KQNNKYYFQTVLNNASFWKEYKFYNANNQELATTVVNDNKKADTRTINVA
VEPGYKSLTTKVHIVVPQINYNHRYTTHLEFEKAIPTLADAAKPNNVKPV
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QTKTQTAHTVKTAQTAQEQNKVQTPVKDVATAKSESNNQAVSDNKSQQTN
KVTKHNETPKQAGPSKDSKAKELPKTG

SEQ ID NO: 2
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IKNPAIKDKDHSAPNSRPIDFEMKKKDGTQQFYHYASSVKPARVIFTDSK
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QNVQPTNEKMTDLQDTKYVVYESVENNESMMDTFVKHPIKTGMLNGKKYM
VMETTNDDYWKDFMVEGQRVRTISKDAKNNTRTIIFPYVEGKTLYDAIVK
VHVKTIDYDGQYHVRIVDKEAFTKANTDKSNKKEQQDNSAKKEATPATPS
KPTPSPVEKESQKQDSQKDDNKQLPSVEKENDASSESGKDKTPATKPTKG
EVESSSTTPTKVVSTTQNVAKPTTASSKTTKDVVQTSAGSSEAKDSAPLQ
KANIKNTNDGHTQSQNNKNT

SEQ ID NO: 3
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KGTKNSAQFEKMVILTENKGYYTVYLNTPLAEDRKNVELLGKMYKTYFFK
KGESKSSYVINGPGKTNEYAY

SEQ ID NO: 4
STSLPTSNEYQNEKLANELKSLLDELNVNELATGSLNTYYKRTIKISGQK
AMYALKSKDFKKMSEAKYQLQKIYNEIDEALKSY

SEQ ID NO: 5
MHHHHHH

SEQ ID NO: 6
LNDIFEAQKIEWHG

SEQUENCE LISTING

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Ser Gln Ala Thr Ser Gln Pro Ile Asn Phe Gln Val Gln Lys Asp Gly
            20 25 30

Ser Ser Glu Lys Ser His Met Asp Asp Tyr Met Gln His Pro Gly Lys
        35 40 45

Val Ile Lys Gln Asn Asn Lys Tyr Tyr Phe Gln Thr Val Leu Asn Asn
    50 55 60

Ala Ser Phe Trp Lys Glu Tyr Lys Phe Tyr Asn Ala Asn Asn Gln Glu
65 70 75 80

Leu Ala Thr Thr Val Val Asn Asp Asn Lys Lys Ala Asp Thr Arg Thr
                85 90 95

Ile Asn Val Ala Val Glu Pro Gly Tyr Lys Ser Leu Thr Thr Lys Val

-continued

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           100                 105                 110

His Ile Val Val Pro Gln Ile Asn Tyr Asn His Arg Tyr Thr Thr His
        115                 120                 125

Leu Glu Phe Glu Lys Ala Ile Pro Thr Leu Ala Asp Ala Ala Lys Pro
    130                 135                 140

Asn Asn Val Lys Pro Val Gln Pro Lys Pro Ala Gln Pro Lys Thr Pro
145                 150                 155                 160

Thr Glu Gln Thr Lys Pro Val Gln Pro Lys Val Glu Lys Val Lys Pro
                165                 170                 175

Thr Val Thr Thr Thr Ser Lys Val Glu Asp Asn His Ser Thr Lys Val
            180                 185                 190

Val Ser Thr Asp Thr Thr Lys Asp Gln Thr Lys Thr Gln Thr Ala His
        195                 200                 205

Thr Val Lys Thr Ala Gln Thr Ala Gln Glu Gln Asn Lys Val Gln Thr
    210                 215                 220

Pro Val Lys Asp Val Ala Thr Ala Lys Ser Glu Ser Asn Asn Gln Ala
225                 230                 235                 240

Val Ser Asp Asn Lys Ser Gln Gln Thr Asn Lys Val Thr Lys His Asn
                245                 250                 255

Glu Thr Pro Lys Gln Ala Gly Pro Ser Lys Asp Ser Lys Ala Lys Glu
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Leu Pro Lys Thr Gly
        275


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<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

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            20                  25                  30

Thr Ser Glu Lys Ala Pro Glu Thr Lys Pro Val Ala Asn Ala Val Ser
        35                  40                  45

Val Ser Asn Lys Glu Val Glu Ala Pro Thr Ser Glu Thr Lys Glu Ala
    50                  55                  60

Lys Glu Val Lys Glu Val Lys Ala Pro Lys Glu Thr Lys Glu Val Lys
65                  70                  75                  80

Pro Ala Ala Lys Ala Thr Asn Asn Thr Tyr Pro Ile Leu Asn Gln Glu
                85                  90                  95

Leu Arg Glu Ala Ile Lys Asn Pro Ala Ile Lys Asp Lys Asp His Ser
            100                 105                 110

Ala Pro Asn Ser Arg Pro Ile Asp Phe Glu Met Lys Lys Lys Asp Gly
        115                 120                 125

Thr Gln Gln Phe Tyr His Tyr Ala Ser Ser Val Lys Pro Ala Arg Val
    130                 135                 140

Ile Phe Thr Asp Ser Lys Pro Glu Ile Glu Leu Gly Leu Gln Ser Gly
145                 150                 155                 160

Gln Phe Trp Arg Lys Phe Glu Val Tyr Glu Gly Asp Lys Lys Leu Pro
                165                 170                 175
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Ile Lys Leu Val Ser Tyr Asp Thr Val Lys Asp Tyr Ala Tyr Ile Arg
            180                 185                 190

Phe Ser Val Ser Asn Gly Thr Lys Ala Val Lys Ile Val Ser Ser Thr
        195                 200                 205

His Phe Asn Asn Lys Glu Glu Lys Tyr Asp Tyr Thr Leu Met Glu Phe
    210                 215                 220

Ala Gln Pro Ile Tyr Asn Ser Ala Asp Lys Phe Lys Thr Glu Glu Asp
225                 230                 235                 240

Tyr Lys Ala Glu Lys Leu Leu Ala Pro Tyr Lys Lys Ala Lys Thr Leu
                245                 250                 255

Glu Arg Gln Val Tyr Glu Leu Asn Lys Ile Gln Asp Lys Leu Pro Glu
            260                 265                 270

Lys Leu Lys Ala Glu Tyr Lys Lys Lys Leu Glu Asp Thr Lys Lys Ala
        275                 280                 285

Leu Asp Glu Gln Val Lys Ser Ala Ile Thr Glu Phe Gln Asn Val Gln
    290                 295                 300

Pro Thr Asn Glu Lys Met Thr Asp Leu Gln Asp Thr Lys Tyr Val Val
305                 310                 315                 320

Tyr Glu Ser Val Glu Asn Asn Glu Ser Met Met Asp Thr Phe Val Lys
                325                 330                 335

His Pro Ile Lys Thr Gly Met Leu Asn Gly Lys Lys Tyr Met Val Met
            340                 345                 350

Glu Thr Thr Asn Asp Asp Tyr Trp Lys Asp Phe Met Val Glu Gly Gln
        355                 360                 365

Arg Val Arg Thr Ile Ser Lys Asp Ala Lys Asn Asn Thr Arg Thr Ile
    370                 375                 380

Ile Phe Pro Tyr Val Glu Gly Lys Thr Leu Tyr Asp Ala Ile Val Lys
385                 390                 395                 400

Val His Val Lys Thr Ile Asp Tyr Asp Gly Gln Tyr His Val Arg Ile
                405                 410                 415

Val Asp Lys Glu Ala Phe Thr Lys Ala Asn Thr Asp Lys Ser Asn Lys
            420                 425                 430

Lys Glu Gln Gln Asp Asn Ser Ala Lys Lys Glu Ala Thr Pro Ala Thr
        435                 440                 445

Pro Ser Lys Pro Thr Pro Ser Pro Val Glu Lys Glu Ser Gln Lys Gln
    450                 455                 460

Asp Ser Gln Lys Asp Asp Asn Lys Gln Leu Pro Ser Val Glu Lys Glu
465                 470                 475                 480

Asn Asp Ala Ser Ser Glu Ser Gly Lys Asp Lys Thr Pro Ala Thr Lys
                485                 490                 495

Pro Thr Lys Gly Glu Val Glu Ser Ser Ser Thr Thr Pro Thr Lys Val
            500                 505                 510

Val Ser Thr Thr Gln Asn Val Ala Lys Pro Thr Thr Ala Ser Ser Lys
        515                 520                 525

Thr Thr Lys Asp Val Val Gln Thr Ser Ala Gly Ser Ser Glu Ala Lys
    530                 535                 540

Asp Ser Ala Pro Leu Gln Lys Ala Asn Ile Lys Asn Thr Asn Asp Gly
545                 550                 555                 560

His Thr Gln Ser Gln Asn Asn Lys Asn Thr
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<210> SEQ ID NO 3
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<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 3

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Lys Met Leu Glu Lys Glu Lys Ala Tyr Lys Glu Ser Phe Lys Asn Ser
20 25 30

Gly Leu Pro Thr Thr Leu Gly Lys Leu Asp Glu Arg Leu Arg Asn Tyr
35 40 45

Leu Lys Lys Gly Thr Lys Asn Ser Ala Gln Phe Glu Lys Met Val Ile
50 55 60

Leu Thr Glu Asn Lys Gly Tyr Tyr Thr Val Tyr Leu Asn Thr Pro Leu
65 70 75 80

Ala Glu Asp Arg Lys Asn Val Glu Leu Leu Gly Lys Met Tyr Lys Thr
85 90 95

Tyr Phe Phe Lys Lys Gly Glu Ser Lys Ser Ser Tyr Val Ile Asn Gly
100 105 110

Pro Gly Lys Thr Asn Glu Tyr Ala Tyr
115 120

<210> SEQ ID NO 4
<211> LENGTH: 84
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 4

Ser Thr Ser Leu Pro Thr Ser Asn Glu Tyr Gln Asn Glu Lys Leu Ala
1 5 10 15

Asn Glu Leu Lys Ser Leu Leu Asp Glu Leu Asn Val Asn Glu Leu Ala
20 25 30

Thr Gly Ser Leu Asn Thr Tyr Tyr Lys Arg Thr Ile Lys Ile Ser Gly
35 40 45

Gln Lys Ala Met Tyr Ala Leu Lys Ser Lys Asp Phe Lys Lys Met Ser
50 55 60

Glu Ala Lys Tyr Gln Leu Gln Lys Ile Tyr Asn Glu Ile Asp Glu Ala
65 70 75 80

Leu Lys Ser Tyr

<210> SEQ ID NO 5
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 5

Met His His His His His His
1 5

<210> SEQ ID NO 6
<211> LENGTH: 14
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<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 6

Leu Asn Asp Ile Phe Glu Ala Gln Lys Ile Glu Trp His Gly
1 5 10

1. A method of determining an *S. aureus* infection in a subject, comprising:

a. generating a plasmablasts-secreted antibody medium by collecting peripheral blood mononuclear cells (PBMCs) from blood in a subject, removing existing antibodies from the PBMCs and culturing the antibody-free PBMCs in a medium for a period of time to allow secretion of antibodies by plasmablasts contained in the PBMCs into the medium;

b. contacting the plasmablasts-secreted antibody medium with one or more *S. aureus* antigens;

c. detecting the presence of one or more antigen-specific antibodies in the plasmablasts-secreted antibody medium at a level above a control; and

d. determining the *S. aureus* infection in the subject based on the detection of the antigen-specific antibodies in step c.

2. The method of claim 1, wherein the one or more *S. aureus* antigens are selected from an IsdA antigen, an IsdB antigen, a CHIPS antigen, and a SCIN antigen.

3. The method of claim 1, wherein the plasmablasts-secreted antibody medium is contacted with one, or two, or three, or all of the IsdA antigen, IsdB antigen, CHIPS antigen, and SCIN antigen.

4. The method of claim 3, wherein the plasmablasts-secreted antibody medium is contacted with all of the IsdA antigen, IsdB antigen, CHIPS antigen, and SCIN antigen.

5. The method of claim 3, wherein the plasmablasts-secreted antibody medium is contacted with three of the IsdA antigen, IsdB antigen, CHIPS antigen, and SCIN antigen.

6. The method of claim 3, wherein the plasmablasts-secreted antibody medium is contacted with two of the IsdA antigen, IsdB antigen, CHIPS antigen, and SCIN antigen.

7. The method of claim 1, wherein the antibody-free PBMCs are cultured for about 36 to about 72 hours.

8. The method of claim 7, wherein the antibody-free PBMCs are cultured for about 72 hours.

9. The method of claim 1, wherein the presence of the one or more antigen-specific antibodies in the plasmablasts-secreted antibody medium is at a level of at least about 10% more than the control level.

10. A kit for determining if a subject has a *Staphylococcus aureus* (*S. aureus*) infection, comprising:

a. one or more antigens selected from the group consisting of an IsdA antigen, an IsdB antigen, a CHIPS antigen, and a SCIN antigen;

b. a reservoir for contacting the one or more antigens with the plasmablasts-secreted antibody medium of claim 1; and

c. a composition for detecting binding between the one or more antigens with the one or more antibodies in the plasmablasts-secreted antibody medium.

11. The kit of claim 10, wherein the one or more antigens are all of the IsdA antigen, IsdB antigen, CHIPS antigen, and SCIN antigen.

12. The kit of claim 10, wherein the one or more antigens are bound to the reservoir in an array.

13. The kit of claim 10, wherein the composition for detecting binding comprises one or more labelled anti-IgG antibodies.

14. The kit of claim 13, wherein the label is fluorescent.

* * * * *

专利名称(译)	用于检测葡萄球菌感染的组合物和方法		
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申请号	US15/563667	申请日	2016-04-04
[标]申请(专利权)人(译)	罗彻斯特大学 埃默里大学		
申请(专利权)人(译)	罗彻斯特大学 埃默里大学		
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摘要(译)

本文提供了用于检测和/或诊断受试者中金黄色葡萄球菌（感染金黄色葡萄球菌）感染的组合物和方法。本发明的令人惊讶的发现是。可以通过使用一种或多种选自IsdA抗原，IsdB抗原，CHIPS抗原和SCIN抗原的抗原来检测金黄色葡萄球菌感染。在一些实施方案中，这些是。金黄色葡萄球菌抗原用于检测来自受试者的浆母细胞分泌的抗体。