



# The correlation between expression of sip protein in different serotypes of group b streptococcus and diagnosis



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## ABSTRACT

Since the surface immunogenic protein (Sip) of group B streptococcus was identified, its immunogenicity and its potential as a universal vaccine candidate has been evaluated extensively. We developed recombinant Sip protein and used it for monoclonal antibody generation to develop immunochromatographic test kit for GBS detection. The test of bacteria and culture media revealed the correlation between Sip protein expression and diagnosis discrepancy, which has never been reported. Furthermore, not only the surface accessibility of the Sip protein may vary from strains or serotypes; the secretion level of Sip protein may also vary.

## 1. Introduction

The bacterium *Streptococcus agalactiae* (*S. agalactiae*), also known as group B streptococcus or GBS, is a commensal bacterium found in gastrointestinal and genitourinary tract of up to 30% of healthy human adults without eliciting clinical symptoms. However, the newborns may become colonized at the time of delivery during passage through the birth canal and can cause serious neonatal infection, such as sepsis, pneumonia, and meningitis (Nuccitelli et al., 2015).

Prenatal screening during the 35th to 37th weeks of gestation are highly recommended (Centers for Disease Control and Prevention, 2010). If a GBS carrier is confirmed, intrapartum antibiotic prophylaxis (IAP) with penicillin or ampicillin will be administered to decrease the incidence of GBS infection in newborns, and it is the currently most accepted strategy for GBS prevention world-wide (Verani et al., 2010). Besides prophylactic IAP, developing vaccination is rather promising to prevent both maternal GBS infection and neonatal GBS infection by functional antibodies which can be transplacentally transmitted. Even better, this strategy can avoid excessive antibiotic administration and antibiotic resistance strains (Kimura et al., 2008). Both successful diagnosis and vaccine development imperatively require a feasible antigen

target.

All clinical isolates of GBS express their type-specific capsula polysaccharide on their surface, and there are ten distinct GBS serotypes, namely Ia, Ib, II, III, IV, V, VI, VII, VIII and IX (Verani et al., 2010). Therefore, using capsula polysaccharide as an antigen target could make the situation fairly complicated to cover all serotypes. The discovery of the ubiquitously surface-exposed Sip protein, short for surface immunogenic protein, overcame the complexity brought by serotype variations.

Comparison of sip genes revealed more than 98% identity, and highly conserved among GBS isolates (Maione et al., 2005). These characters make Sip to be a promising target protein for both diagnosis and a universal vaccine candidate, therefore, the immunogenicity and protection capability of Sip have been extensively studied (Brodeur et al., 2000; Maione et al., 2005; Martin et al., 2002; Xue et al., 2010).

In this study, we developed recombinant Sip (rSip) using serotype Ia bacteria genome DNA as a template, and then used this rSip for immunization to develop monoclonal antibodies (McAbs). The antibodies derived from this rSip exhibited good sensitivity and specificity. They were able to detect both rSip and nature Sip protein, and the latter included the localized form on the surface of GBS cells and secreted form

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in culture medium.

## 2. Materials and methods

### 2.1. Cloning of sip gene and his-tagged rSip expression, purification and identification

Chromosomal DNA was extracted from *S. agalactiae* strain TW3 using Promega DNA extraction kit (Promega, Beijing, China). According to the sequence of sip gene from *S. agalactiae* (tilapia origin; HQ878436.1, Genbank), primers were designed as 5'-GCCGGATCCATGGAATGAA-TAAAAAGGTAC -3' and 5'-GCGGTCGACTTAGTAAAGGA-TACGTGAACGTGG -3', containing BamHI and Sall restriction sites respectively, incorporated at the 5' ends of the primers to facilitate cloning. PCR was carried out under the following conditions. After initial denaturation of DNA at 94 °C for 5 min with a thermal cycler, the program was set at 94 °C for 30 s, 55 °C for 35 s, and 72 °C for 1 min 18 s for a total of 30 cycles and then at 72 °C for 10 min for the final extension. Amplified sip DNA was ligated to the pET32a vector containing a His-Tag sequence. The resulting recombinant plasmid was named pET32a-sip, which was transformed into *Escherichia coli* cells BL21 to generate sufficient rSip protein to immunize the mice required for McAb production. The transformants were cultured in LB broth containing 100 µg/mL of kanamycin at 37 °C for 24 h. The expression of His-tagged rSip was induced by adding 0.8 mM isopropyl-beta-D-thiogalactopyranoside (IPTG). The fusion rSip protein in the bacterial cell lysate was purified using immobilized metal ion affinity chromatography (IMAC) with TALON superflow resin (GE, Beijing, China) following the manufacturer's instructions. The eluted protein concentration was quantitated by standard BCA assay.

Protein identification was performed by peptide mass fingerprinting (University of Manitoba, Winnipeg, Canada). The rSip was firstly digested with trypsin, and the tryptic peptides were sequentially analyzed by tandem mass spectrometry (matrix-assisted laser desorption-ionization-time of flight mass spectrometer, MALDI-TOF/TOF). The mass spectrum database searches were undertaken using MASCOT software (Matrix Science) to search in the National Center for Biotechnology Information (NCBI) protein database (NCBIprot, 20180727).

### 2.2. GBS isolates and growth conditions

Group B *Streptococcus* isolates used in this study were ATCC1138 (Serotype Ia), ATCC12401 (Serotype Ib), ATCC13813 and ATCC-BAA2675 (Serotype II), ATCC12403 and ATCCBAA22 (Serotype III) and ATCCBAA2673 (Serotype IV), ATCCBAA611 (Serotype V) and two fish origins TW3 (serotype Ia) and TW7 (serotype Ia). GBS isolates were grown in brain heart infusion (BHI) broth at 37 °C with shaking (200 rpm).

### 2.3. SDS-PAGE and western blotting

Crude GBS whole cell (WC) extracts, purified rSip or nature Sip were subjected to 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblots. For WC samples, a 10 ml overnight culture was centrifuged to obtain the pellet, which was added with 25 µl of sample buffer (0.2 M Tris-HCl (pH 6.8), 1% SDS, 2% mercaptoethanol, 10% glycerol, 0.001% bromophenol blue) and then boiled for 5 min, and after centrifugation, 10 µl of supernatant was applied to the gel. After electrophoresis, protein bands were visualized by staining with coomassie brilliant blue (CBB) or transferred to nitrocellulose membranes for western blotting method using anti-rSip McAbs. For western blotting, the membrane was treated as follows: blocked with phosphate-buffered saline (PBS, pH 7.4) containing 3.0% BSA (Sigma-Aldrich, Beijing, China) and 0.1% Tween 20 (Sigma-Aldrich, Beijing, China) for 1 h at 24 °C, washed 3 times with PBS containing 0.1% Tween 20, incubated with 100 ng/ml of anti-rSip antibodies at 4 °C for 1 h, washed 3

times with PBS containing 0.1% Tween 20, incubated with 2,000-fold-diluted horseradish peroxidase (HRP)-conjugated anti-mouse IgG (Sigma-Aldrich, Beijing, China) at 24 °C for 1 h, washed 3 times with PBS containing 0.1% Tween 20, and then soaked in the TMB membrane peroxidase substrate system (Sigma-Aldrich, Beijing, China) for color development.

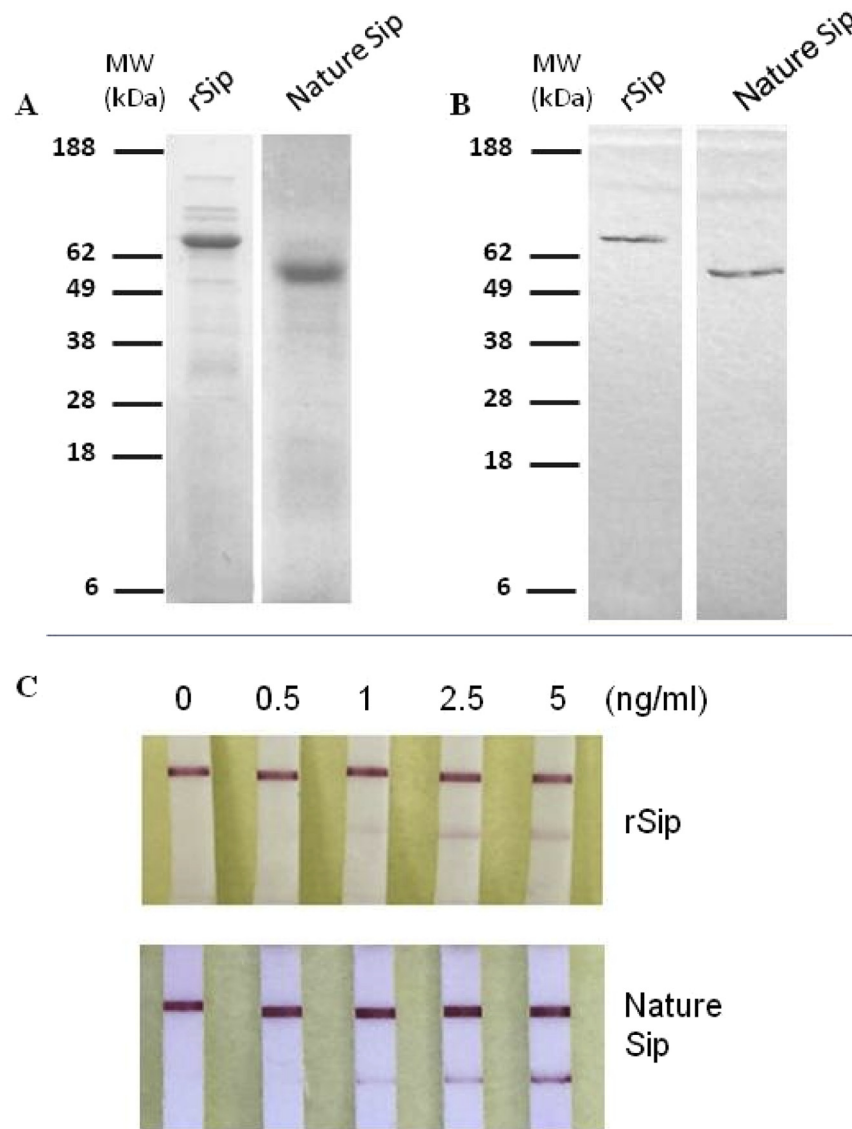
### 2.4. Monoclonal antibody preparation and characterization

Monoclonal antibodies were obtained by a previously described method using rSip as immunogen (Chen et al., 2014). In short, the 6 to 8-week-old BALB/c female mice were given four intraperitoneal injection of rSip at 2-week intervals, and the immune response was assessed by measuring the titer of polyclonal antibody in mice sera using indirect ELISA. The immunized mice with the highest titer were used as a spleen cells donor in hybridoma production. The desired cell lines were recloned three times by limiting dilution using spleen cells from a non-immune BALB/c mouse as feeder cells to achieve monoclonality and stability. For large scale production of ascites, the 8-week old female BALB/c mice, primed with liquid paraffin, were injected intraperitoneally with  $1.0 \times 10^6$  hybridoma cells per mouse, and the ascetic fluid was drained with a 12-gauge needle after a few weeks. The ascites were centrifuged at 8000 g at 4 °C for 10 min to remove cells, and the collected supernatants were precipitated in 50% saturated ammonium sulfate (pH 7.4), followed by extensive dialysis against 0.02 M phosphate buffer (pH 7.4) at 4 °C. The dialyzed solution was further purified by protein A affinity chromatography to achieve high quality.

The purity of the McAbs was assessed by 12% SDS-PAGE. The specificity of the McAbs was evaluated by western blotting or ICT analysis. The cross-reactivity was assessed using the following microorganisms: *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Aeromonas caviae*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Aeromonas hydrophila*, *Bacillus subtilis*, *Vibrio alginolyticus*, *Enterobacter cloacae*, *Shewanella putrefaciens* and *Klebsiella pneumoniae*, *Neisseria gonorrhoeae*, *Stenotrophomonas maltophilia*, *Proteus mirabilis*, *Candida tropicalis*, *Salmonella typhi*, *Salmonella paratyphi*, *Serratia marcescens*, *Escherichia coli*, *Candida albicans*, *Citrobacter freundii*, *Pseudomonas aeruginosa*. The titer of McAbs was determined by indirect ELISA. The immunoglobulin subclass was determined by a Mouse Monoclonal Antibody Isotyping Reagents following manufacturers' instructions (Sigma-Aldrich, Beijing, China).

### 2.5. Immunoaffinity purifications (IAP) and characterization of nature GBS sip protein

Purified McAb CC11 (25 mg) and normal mouse IgG (11.2 mg) were coupled to CNBr-activated Sepharose 4B (GE, Beijing, China) as described by the manufacturer. Approximately  $10^{10}$  bacteria culture (*S. agalactiae* strain TW3) were sonicated in 100 ml lysis buffer (20 mM Tris, 5 mM EDTA, 1% Triton X-100, pH 7.4) for 3 min at room temperature and incubated for 45 min at 4 °C. Cell debris was removed by centrifugation (30 min, 13,000×g, 4 °C). Prior to chromatography columns were equilibrated with 100 ml of wash buffer (50 mM Tris, 500 mM NaCl, 0.02% Na<sub>3</sub>, 0.1% Triton X-100, pH 8.0). The 100 ml lysate was passed through the mouse IgG column and then through the McAb CC11 column at a flow rate of 0.2 ml/min. Unbound proteins were removed by extensive washing with 150 ml wash buffer and 10 ml of 150 mM NaCl. McAb CC11-bound protein was eluted with 50 ml of acidic buffer (500 mM NaCl, 200 mM acetic acid, 0.1% Triton X-100, pH 2.8) at a flow rate of 0.5 ml/min. Columns were then equilibrated with 40 ml of 100 mM Tris-HCl buffer (pH 7.8) and 10 ml of 150 mM NaCl. Remaining protein was then eluted with 40 ml of alkaline buffer (50 mM diethylamine, 0.1% Triton X-100, pH 11.5). Fractions (4 ml) were collected and immediately neutralized (1 ml of 1 M Tris buffer, pH 9). Protein containing samples were subjected to SDS-PAGE. The eluted protein concentration was



**Fig. 1.** Sip protein detection in SDS-PAGE, western blotting and ICT strips. Panel A: purified rSip and nature Sip protein migrating on SDS-PAGE; Panel B: purified recombinant Sip and nature Sip protein reacted to the pool of sera from whole cell immunized mouse; Panel C: The detection limit of ICT strips for recombinant Sip and nature Sip protein. Please refer to the supplementary data for the non-adjusted images.

quantitated by standard BCA assay.

## 2.6. Preparation ICT strips and evaluation

ICT strips were prepared as previously reported (Chen et al., 2014) using NE1a as coating antibody and CC11 as colloid gold conjugated labeling antibody. To perform a test, after fully contacted with sample solution, the strips were kept flat at room temperature for 5 min to develop test results. Either free Sip protein (rSip, nature Sip) or Sip protein on the surface of GBS cells will interact with the colloid gold conjugated antibodies. The antigen-antibody-gold complex will migrate towards the test region where it will be captured by immobilized coating antibodies, forming a visible pink line. To serve as an internal control, a control line should always appear in the control region after the test is completed. Visible bands appeared at both the test and control region represented a positive test result. Only one red band at the control region represented a negative test result. Regardless whether there was a line at the test region, the absence of a line at the control region indicated the test was invalid and the test should be repeated.

To determine the detection limits of GBS whole cell or Sip protein, the

rSip protein, nature Sip and GBS bacteria cultures were serially diluted and tested by ICT strips. For rSip or nature Sip, they were serially diluted at 10-fold from 10 ng/ml to 0.1 ng/ml. For GBS bacteria cultures, different serotypes of strains were cultured in 20 ml BHI broth for 24 hours and the culture products were then centrifuged to separate supernatant and cell pellet. The WC pellets were resuspended in 20 ml PBS, and washed twice, then serially diluted at 10-fold from  $10^9$  to  $10^3$  cfu/ml for testing. The cross-reactivity of ICT strips was evaluated by examining the interfering microorganisms explored in western blotting at  $1.0 \times 10^{10}$  cfu/ml.

## 2.7. Sample preparation and acquisition for surface sip expression

Once  $OD_{600nm}$  1.0 was reached, bacteria cultures were centrifuged at  $4000 \times g$  for 5 min to pellet the bacteria. The pellet was re-suspended in the same volume of PBS containing 2% formaldehyde and incubated at room temperature for 1 h for fixation. Bacteria were then suspended and washed in PBS followed by centrifugation at  $4000 \times g$  for 5 min for three times. The final cell pellet was resuspended in 1 mL PBS, which was stored at 4 °C before use.

The expression of Sip on the surface of whole GBS bacteria was measured on formaldehyde-fixed GBS using a flow cytometric assay performed in 96-well microtitration plates. Briefly, 2  $\mu$ l of purified McAb 100 ng/ml was added to 198  $\mu$ l of serotype Ia, Ib, II, III, IV or V GBS bacteria at  $5 \times 10^7$  cfu/mL in blocking buffer (1% BSA in PBS). This was incubated at 25 °C for 30 min with shaking (900 rpm), then pelleted. Supernatant was removed and the pellet was washed twice with 200  $\mu$ l of blocking buffer. Alexa Fluor® 488 Goat Anti-Mouse IgG (H&L) (Abcam) (1:500) in blocking buffer was added and washed twice more with blocking buffer. Samples were incubated for 20 min at 4 °C before analyzed by flow cytometer.

Assays were analyzed using a Beckman Coulter Cyan flow cytometer equipped with a Cytex 96-well microtitre plate reader. For each sample, 10,000 individual events were analyzed for fluorescence. A fluorescence index (FI) was calculated for each sample as the mean fluorescence of each specific strain. The final result for each test was expressed as the average FI of triplicate test samples minus the average FI of the bacteria and conjugate-only control, plus/minus standard deviation of three experiments per group. All statistical calculations were performed with SPSS20.0 statistical software (IBM, Armonk, NY). Comparisons between groups were analyzed by one-way ANOVA followed by a Duncan's post hoc test. *P* values <0.05 were considered statistically significant.

### 2.8. Analyze the secreted form of sip

For western blotting analysis, 25  $\mu$ l supernatant of each centrifuged GBS strain culture described above was subjected to SDS-PAGE directly without further processing. The electrophoresis, transfer and detection were performed as described above in SDS-PAGE and Western blotting section. The confirmed detectable whole cell lysate was employed for the positive control.

For ICT strips analysis, the supernatant of each GBS strain culture described above was serially diluted at 2-fold and tested by strips. The final detectable dilution factor for each strain was recorded accordingly.

## 3. Results

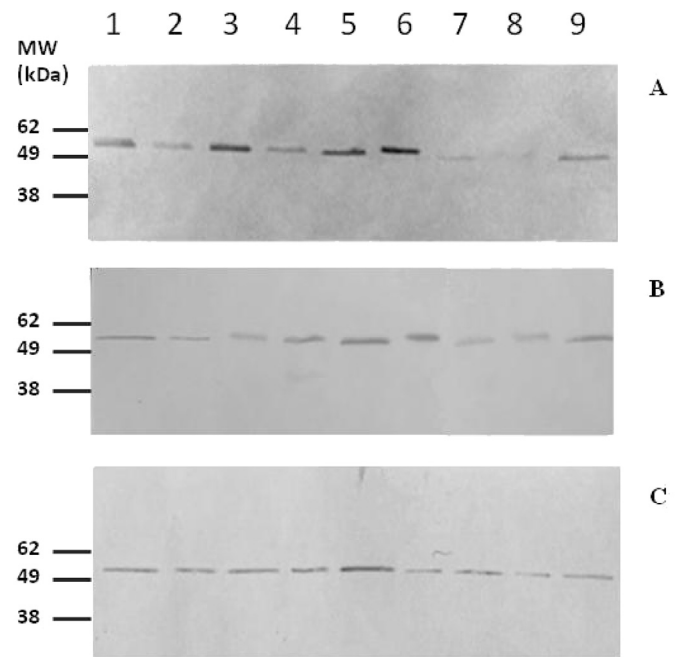
### 3.1. Production of the rSip and nature sip

The rSip protein purified by IMAC strategy with a nickel-charged resin column migrated at an approximate molecular mass of 72 kDa (Fig. 1A) on SDS-PAGE with the purity of >90%. The MASCOT search result further confirmed the major component of this recombinant protein was sip protein (Score: 12872; GenBank Name: AEK06226.1). The nature Sip protein purified by IAP strategy migrated at an approximate molecular mass of 53 kDa (Fig. 1A) on SDS-PAGE with the purity of >90%. The observed difference between rSip and nature Sip protein was generated from a few amino acid sequences inherited in pET-32 vector, including Trx•Tag, His•Tag and S•Tag etc. The MASCOT search also confirmed that Trx•Tag thioredoxin protein (Score: 1274; NCBI Reference Sequence: WP\_054575894.1) was the second major component of recombinant Sip.

Both materials reacted to the pooled sera from whole cell immunized mice (Fig. 1B), which confirmed their immunoreactivity.

### 3.2. Preparation anti-sip McAbs

Two hybridoma cell lines, CC11 and NE1a, were established from mice spleen cells immunized with rSip and each produced McAb was reactive with Sip. They were produced in large scale through ascitic fluid as described in section 2.4. The collected ascites was then purified in two sequential steps, namely ammonium sulfate precipitation and protein A affinity chromatography. The purified antibodies were named as McAb CC11 and McAb NE1a respectively. Both final products had purity over 95% (examined by SDS-PAGE) and titer over  $10^{-6}$  (examined by indirect ELISA). The subtypes of McAb CC11 and McAb NE1a were IgG<sub>2b</sub> and IgG<sub>1</sub>



**Fig. 2.** Different serotypes of GBS tested by the Western blotting method. Panel A and B represented using CC11 and NE1a as primary incubation antibody respectively to detect GBS whole cell lysate (1: ATCC BAA1138; 2: ATCC 12401; 3: ATCC BAA2675; 4: ATCC 12403; 5: ATCC BAA22; 6: native Sip; 7: ATCC BAA2673; 8: ATCC BAA611; 9: TW3). Panel C represented using CC11 as primary incubation antibody to detect secreted Sip protein in cell culture media (1: ATCC BAA1138; 2: ATCC 12401; 3: ATCC BAA2675; 4: ATCC 12403; 5: ATCC BAA22; 6: ATCC BAA2673; 7: ATCC BAA611; and 8: TW3). Lane 9 was TW3 cell lysate as a positive control. Please refer to the supplementary data for the non-adjusted images.

**Table 1**

The whole cell detection limit (CFU/ml) and culture supernatant dilution factors of different serotypes of GBS.

Serotype	Culture Supernatant Dilution factor	Whole Cell Detection limit (CFU/ml)	
ATCC 1138	Ia	1:64	$1.5 \times 10^6$
ATCC 12401	Ib	1:64	$1.0 \times 10^6$
ATCC 13813	II milk	1:32	$1.0 \times 10^6$
ATCC BAA2675	II	1:32	$1.5 \times 10^6$
ATCC 12403	III	1:64	$1.5 \times 10^6$
ATCC BAA22	III	1:128	$1.0 \times 10^6$
ATCC BAA2673	IV	1:64	$4.0 \times 10^6$
ATCC BAA611	V	1:64	$3.5 \times 10^6$
TW3	Ia fish	1:32	$1.5 \times 10^6$
TW7	Ia fish	1:32	$1.5 \times 10^6$

respectively.

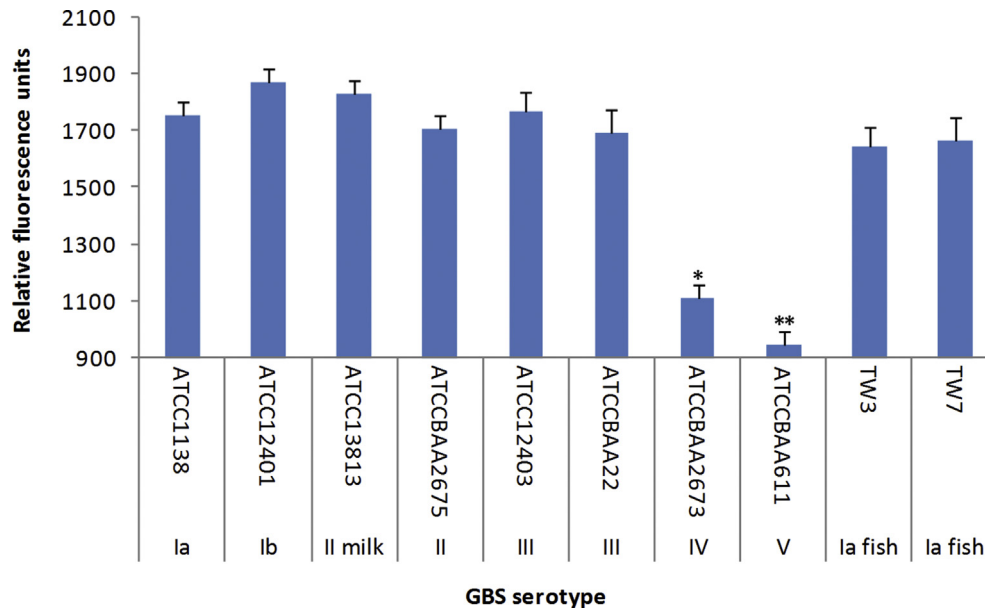
### 3.3. Evaluations of anti-sip McAbs and ICT strips

Western blotting analysis demonstrated clearly that both McAb CC11 and McAb NE1a could specifically detect different serotypes of GBS strains (Figs. 2A and 2B) and did not cross-react to cell extracts from other potential interfering bacteria strains (data not shown).

The detection limits of ICT strips made by McAb CC11 and NE1a were 1 ng/ml for both rSip protein and nature Sip protein (Fig. 1C). When testing intact GBS bacteria, the ICT strips showed various detection levels. Most of the serotypes could be detected at  $1.0$ – $1.5 \times 10^6$  cfu/ml, except ATCC BAA2673 (serotype IV) ( $4.0 \times 10^6$  cfu/ml) and ATCC



**Fig. 3.** Specificity evaluation using ICT strips. ICT strips composed by matched antibody pair CC11 and NE1a detected whole cell bacteria, including interference bacteria and a positive control (Two bands appeared at both the test and control region, namely T line and C line respectively, represents a positive test result. Only C line appeared represents a negative test result). 1: *S. agalactiae* TW3, 2: physiological saline (negative control), 3: *Enterococcus faecalis*, 4: *Streptococcus pneumoniae*, 5: *Streptococcus iniae*, 6: *Streptococcus pyogenes*, 7: *Aeromonas caviae*, 8: *Salmonella typhimurium*, 9: *Staphylococcus aureus*, 10: *Vibrio cholerae*, 11: *Vibrio parahaemolyticus*, 12: *Aeromonas hydrophila*, 13: *Bacillus subtilis*, 14: *Vibrio alginolyticus*, 15: *Enterobacter cloacae*, 16: *Shewanella putrefaciens*, 17: *Klebsiella pneumoniae*, 18: *Neisseria gonorrhoeae*, 19: *Stenotrophomonas maltophilia*, 20: *Proteus mirabilis*, 21: *Candida tropicalis*, 22: *Salmonella typhi*, 23: *Salmonella paratyphi*, 24: *Serratia marcescens*, 25: *Escherichia coli*, 26: *Candida albicans*, 27: *Citrobacter freundii*, 28: *Pseudomonas aeruginosa*.



**Fig. 4.** FACS analysis of Sip expression in different GBS serotypes. Different serotypes of GBS strains were grown and processed as described in Material and Methods. A fluorescence index (FI) was calculated for each sample as the mean fluorescence of each specific strain. Shown are mean values and standard deviations of three independent experiments (\* $p < 0.05$ . \*\* $p < 0.01$ ).

BAA611 (serotype V) ( $3.5 \times 10^6$  cfu/ml) (Table 1). No cross-reactivity was observed to interfering microorganisms at  $1.0 \times 10^{10}$  cfu/ml (Fig. 3).

### 3.4. Sip expression on cell surface and in culture media

FACS analysis (Fig. 4) revealed that ATCC BAA611 (serotype V) and ATCC BAA2673 (serotype IV) had the significantly low expression of Sip on cell surface among the group. The highest expression candidate, ATCC 12401 (serotype Ib), expressed almost twice of the Sip protein on cell surface than ATCC BAA611. All the rest candidates expressed similar level of Sip protein without significant difference.

Different serotypes of GBS strains showed various expression level of secreted form of Sip in culture media too. ICT strips applied on serial dilution test demonstrated dilution factor ranged from 1:32 to 1:128 from serotype to serotype (Table 1). ATCC BAA22 (serotype III) secreted the highest level of Sip protein. This fact was also revealed by western blot (Fig. 2C, Lane 5).

## 4. Discussion

Transmissions of GBS from carrier women to neonates during delivery can cause severe neonatal morbidity and mortality. Although promising vaccination may ultimately reduce the incidence of GBS infection, the development of an effective vaccine and identifying potential vaccine candidates against this pathogen is still underway. Up to

date, IAP treatment based on screening for pregnant women at 35–37 weeks of gestation is highly recommended. Therefore, current effort still focuses on cost-effective rapid test for screening early-onset GBS infections.

Sip is a surface protein with an approximate molecular mass of 53 kDa, identified by a group of Canadian scientists more than a decade (Brodeur et al., 2000). This protein is highly conserved in GBS and ubiquitously presented on the surface of bacteria. Therefore, Sip has been recognized as a promising candidate for both diagnosis and vaccination purpose. Protective effect from GBS infection by maternal immunization with recombinant Sip protein (Martin et al., 2002; Xue et al., 2010) as well as the development of applicable rapid diagnosis tools (Matsui et al., 2013) was studied by different research groups.

Edmond and colleagues reviewed sero-epidemiological studies data and concluded that serotype III accounted for almost half of all isolates in the global serotype distribution, followed by serotypes Ia, Ib, II, and V, which together accounted for more than 85% of serotypes in all regions (Edmond et al., 2012). All these major pathogenic GBS serotypes were carefully examined and tested in this study, including two fish origins as it is also one of the major pathogens associated with streptococcosis outbreaks in fish farms worldwide.

The ICT strips composed by McAb pairs developed in this study could detect rSip protein and nature Sip protein both at 1 ng/ml, as well as approximate  $10^6$  cfu/ml GBS pure cultured cells for multi-serotypes. In addition, a wide variety of bacteria/fungi strains were tested by

manufactured ICT strips to verify its cross-reactivity, including other major members from streptococcus family and microorganisms commonly found in human gastrointestinal and urogenital tract, as well as some aquatic bacteria. The recruited candidates were either pathogenic or existing as normal microbial flora. The results validated that no cross-reaction was noticed at  $10^{10}$  cfu/ml. Therefore, McAbs and ICT strips developed in this study presented competitive sensitivity and specificity for clinical use.

Interestingly, detection limit discrepancies were noticed when testing different serotypes of strains, which may range from  $1.0 \times 10^6$  cfu/ml to  $4.0 \times 10^6$  cfu/ml. This has been also noticed by another study of immunochromatographic detection of the GBS antigen from enrichment cultures (Matsui et al., 2013).

In a multiple genome screen study for identifying a universal GBS vaccine candidate, Maione and colleagues also demonstrated that surface accessibility of sip antigen may vary from strain to strain depending on the presence of polysaccharide capsule, even though the antigen coding gene is conserved among different serotypes (Maione et al., 2005). We assumed the accessibility of sip antigen to our ICT strips is correlated to its expression level as well, so we performed FACS analysis to indirectly examine the Sip expression on cell surfaces from different serotypes. The results supported our hypothesis that the accessibility of Sip on cell surface was positively correlated to the detection limit of ICT strips. The lower expression of Sip on bacteria led to the relatively more difficult detection by ICP strips, e.g. ATCC BAA2673 and 611 strains, although the correlation was non-linear.

Not only can be found on cell surface, Sip can also be found in GBS culture supernatant (Brodeur et al., 2000), which has been confirmed in this study as well. But more interestingly, the data suggested that secretion of Sip protein was also diverse from strain to strain. In our study, the culture supernatant dilution factor determined by ICT strips ranged from 1:32 (ATCC 13813 (II), ATCC BAA2675 (II), TW3 (Ia) and TW7 (Ia)) to 1:128 (ATCC BAA22 (III)), indicating that the secretion level of sip may vary by serotypes. The western blotting analysis also reinforced the evidence that ATCC BAA22 (III) had the highest secretion level among tested strains, although data was not quantitated.

The mechanisms of this serotype-specific expression level and how it may influence on individual pathogenicity are still largely unknown. However, the impact on diagnosis accuracy should not be neglected. Better and profound understanding of this highly conserved protein target is inevitable and imperative for further employing this promising candidate for vaccination and diagnosis purpose.

## Declarations

### Author contribution statement

Shiliang Cheng, Jiae Han: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Gang Lu, Yidan Huang, Qin Yan: Analyzed and interpreted the data;

Wrote the paper.

Zuwei Yuan: Analyzed and interpreted the data.

Guanjun Huang, Jian Zheng, Tianqiang Liu: Contributed reagents, materials, analysis tools or data.

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### Competing interest statement

The authors declare no conflict of interest.

### Additional information

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