A POCT to Rapid Detect GBS with Highly Sensitivity

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Abstract: Group B streptococcus (GBS) is a leading cause of invasive neonatal infections and a significant pathogen in immunocompromised adults. Screening of GBS colonization in pregnant women determines the need for antibiotic prophylaxis in that pregnancy. Therefore, efficient and rapid determination of the GBS colonization status of pregnant women is crucial. Here, we set up a POCT with specific spectral absorption of chromogenic culture media to replace the traditional visual identity of GBS, which greatly improved the sensitivity of GBS detection, and decreased the time to identify it.

1 INTRODUCTION

Group B streptococcus (GBS) is a Gram-positive encapsulated bacterium that belongs to the group of pyogenic streptococci, and an asymptomatic colonizer of the digestive and genitourinary tracts of healthy human adults. However, it can cause severe invasive infections in neonates and immunocompromised adult patients. In 1960s, GBS was identified into a leading cause of life-threatening neonatal infections(Hood et al., 1961; Rosa-Fraile and Spellerberg, 2017).

In neonatology, there are two distinguishable clinical syndromes: one is called as early-onset disease (EOD), in which GBS infection occurs within the first week of life (especially within the first 24 h); another is called as late-onset disease (LOD), in which GBS infection presents after 7 to 90 days postpartum. EOD is caused by vertical transmission through either ascending infection from the genital tract or during labor and birth. Numerous studies have shown that up to 30% of pregnant women worldwide are colonized with GBS, and vertical transmission occurs for roughly 50% of colonized mothers. About 1% of colonized newborns develop EOD, which may be in connection with ruptured membranes because the infection of the fetus can happen only it exposed in GBS. Bacteremia without a focus is the most common clinical syndrome, followed by pneumonia and meningitis. Even today the case fatality rate for EOD is estimated to be 2 to 10%, and fatal outcomes are more frequent among premature neonates(Edwards et al., 2016).

Most EOD is due to the contact of the neonate with GBS during delivery, therefore, intrapartum antibiotic prophylaxis (IAP) administered to GBS carriers prevents vertical transmission in the vast majority of cases, and its widespread use has resulted in significant reductions in the incidence of EOD. LOD, however, is most likely acquired from breast milk, or from nosocomial or community sources. Prematurity is the main risk factor for developing LOD, and bacteremia without a focus of infection is the most common presentation. The mortality rate for LOD is lower, but meningitis and subsequent sequelae are more frequently associated with LOD(Verani et al., 2010).

GBS also causes significant maternal morbidity, including endometritis, chorioamnionitis, bacteremia, and postpartum wound infections. GBS urinary tract infections are associated with miscarriages, preterm births, and low-birth-weight newborns. Although GBS seldom causes disease in healthy adults, it is responsible for serious infections in diabetics, elderly individuals, residents in nursing homes, and otherwise immunocompromised patients. The successful administration of IAP and the treatment of severe GBS infections rely on efficient and reliable detection of GBS in clinical samples(Edwards and Baker, 2005).

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In recent years, there has been rapid expansion in the availability of chromogenic media for the detection of GBS. These culture media contain enzyme substrates linked to indoxyl chromogens, and the target microorganisms are characterized by specific enzyme systems that metabolize the substrate, resulting in release of the chromogen. Subsequently, the indigoid dye formed upon oxidation and dimerization of indoxyl molecules in the presence of oxygen precipitates within the colonies, leading to typical brightly contrasting colors(Orenga et al., 2009). Therefore, the amplitude of the contrasting is positively to concentration of GBS. However, the traditional visual identity usually need at least 72 h to determine whether the GBS colonize, thus, a rapid highly sensitive detection of GBS is urgent.

2 MATERIALS AND METHODS

2.1 A POCT Based on the Specific Spectral Absorption of Chromogen

We developed a POCT (a point-of-care testing(Shu and Chen, 2022)) based on chromogenic media to rapid detect GBS, which meet the clinical needs with highly sensitivity and rapid detection.

Figure 1 shows the mechanism of detection of the POCT device. A parallel excitation light with a wavelength of λ_1 is filtrate from filter 1, and then incident to the sample by perpendicular to the bottom of tube. As a result, the irradiated sample emits another light (λ_2), which is the so-called emission light, however, partial λ_2 light will be absorbed by indoxyl chromogen, and the rest emission light will be finally detected by PMT through filter 2. The absolute detected value of time progress is directly displayed in Fig.2.

The absorbed amount of light λ_2 is proportional to the concentration of indoxyl chromogen, while the latter is also proportional to the concentration of GBS. Thus, the measurable absorbed amount of light λ_2 is indirectly proportional to the concentration of GBS, that is, the concentration of GBS (c) can be quantitatively measured by the absorbed amount of light λ_2 ($Q_0 - Q_i$) as defined in Eq.(1).

2.2 Preparation of Samples and Measuring

Two tubes of culture media with 1.2 mL were supplied by Wenzhou Beikete Medical Equipment Co., Ltd. One contains indoxyl chromogens with higher concentration and is labeled “5”, another is without

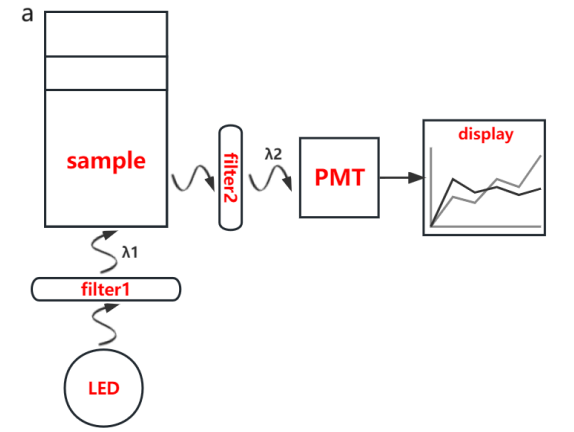

Figure 1: **a:** The mechanism of the POCT. LED is the source of excited light. A parallel excitation light with a wavelength of λ_1 is filtrate from filter 1, and then incident to the sample by perpendicular to the bottom of tube. As a result, the irradiated sample emits another light (λ_2), which is the so-called emission light, however, partial λ_2 light will be absorbed by indoxyl chromogen, and the rest emission light will be finally detected by PMT through filter 2. The absolute value of time progress is directly displayed in Fig.2. **b:** The sample of POCT in experiment.

any chromogens and labeled “0”. Fig.2 shows the samples of chromogenic media with different concentration of the mixture of chromogens and corresponding measured absolute light intensity, which is prepared and measured simultaneously as follows:

Firstly, We measured the value of the emission light from tube “0”, and the platform “0” (the first higher platform from right) in Fig.2 is its time process;

Secondly, a 5 μL samples of “5” is injected in tube “0”, the new sample is labeled “1”, and the measured value is shown in platform “1” (the second higher platform from right) in Fig.2;

Thirdly, Another 5 μL samples of “5” is injected in tube “1”, the new sample is labeled “2”, and the measured value is shown in platform “2” (the third higher platform from right) in Fig.2;

Fourthly, A 10 μL samples of “5” is injected in tube “2”, the new sample is labeled “3”, and the

measured value is shown in platform "3" (the fourth higher platform from right) in Fig.2;

Fifthly, A 20 μL samples of "5" is injected in tube "3", the new sample is labeled "4", and the measured value is shown in platform "4" (the fifth higher platform from right) in Fig.2;

Finally, We measured the value of the emission light from tube "5", and the platform "5" (the sixth higher platform from right) in Fig.2 is its time process;

The normalized concentrations of chromogens of sample "0∼5"(with respect to that of the sample "5"), c, approximate to 0, 4.15×10^{-3}, 8.26×10^{-3}, 1.64×10^{-2}, 3.23×10^{-2}, and 1.00 respectively.

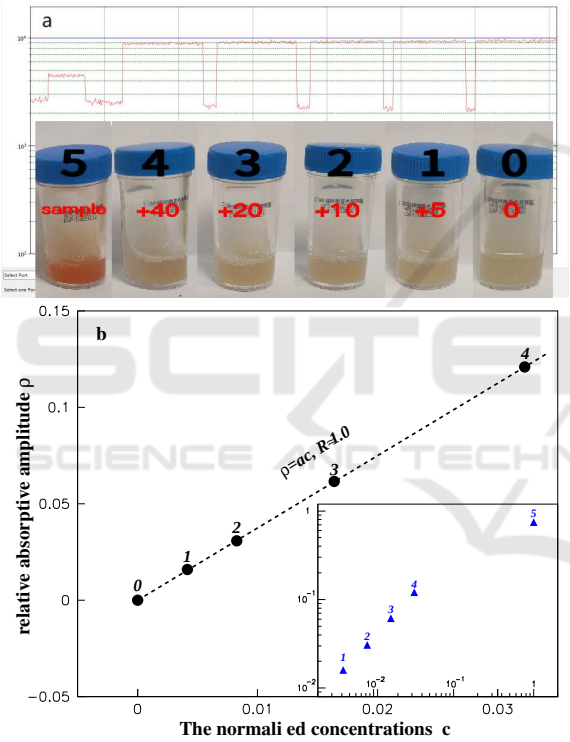

Figure 2: **a:** The measured absolute light intensity of samples by our POCT vs time. Each higher platform from *right* to *left* corresponds to the value of sample 0,1,2,3,4 and 5 (**The inserted panel**) respectively, while the lower platform is the background value, so that the absolute light intensity is the higher one minus the lower one. The relative absorptive amplitude of sample i, ρ_i, can be defined by Eq.(1), and shown in bottom. **b:** The relative absorptive amplitude (ρ) vs normalized concentrations of samples (c), which is almost linear ($\rho = ac$, where $a(= 3.75)$ is the fitted constant) at lower concentrations with regression coefficient(R) of 1.0. **The inserted panel** displays that the ρ_5/ρ_1 is nearly 10^2, which means our POCT is very much more sensitive than the traditional visual one.

3 RESULTS

The absolute intensity of the measured emission light (Q_i) equals the value of higher platform minus the background noise (corresponds the value of lower platform). Thus, the absorptive amplitude of sample is $Q_0 - Q_i$, where Q_0 is supposed the value that there is not any indoxyl chromogens in chromogenic media such as sample "0".

The relative absorptive amplitude of sample "i", ρ_i, can be defined by

$$\rho_i \equiv 1 - \frac{Q_i}{Q_0}, \tag{1}$$

and shown in Fig.2. The relation between the relative absorptive amplitude (ρ) and the normalized concentrations of samples (c) is almost linear ($\rho = ac$, where $a(= 3.75)$ is the fitted constant) at lower concentrations with regression coefficient(R) of 1.0. The value of samples "1" $\sim$ "4" is obviously higher than "$\rho_0(\equiv 0)$", which means that the sensitivity of our method is much higher than that of the traditional visual one as shown in Fig.2. The enormous quantitative difference between samples "1" and "5" furthermore displays the advance of the POCT. It is possible for chromogenic media to greatly decrease identification time if our POCT is loaded.

4 CONCLUSIONS AND DISCUSSION

The sensitivity of screening methods based on the culture identification of maternal carriage of GBS depends on the timing of specimen collection, the source of the specimen, and the culture technique used by the microbiology laboratory. The chromogenic media is a good alternative for the identification of GBS carrier status among near-term pregnant women. Decreasing identification time of GBS as well as improving sensitivity is our motivation to develop the POCT.

It is very difficulty for the traditional visual identity to determine whether GBS colonized in samples 1 $\sim$ 4 as shown in Fig.2. However, our POCT achieves a rapid detection of GBS, and reinforced the advance of chromogenic media. Each platform of time course displays difference in altitude in Fig.2, which means our POCT is more sensitive than the traditional visual one.

It has to be pointed that a quantitative measurement of the number of GBS in sample "5" should be done, so that the relation between ρ and number of GBS can be determined, because it is important for clinical trial to decide the "threshold" of positive/negitive.

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