Electronic Supplementary Material (ESI) for Analyst.

Supplementary information

Rapid Detection of Group B *Streptococcus* (GBS) from artificial urine samples based on IFAST and ATP Bioluminescence Assay: from development to practical challenges during protocol testing in Kenya

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Abstract: We report the rapid detection (20 min) of *Streptococcus agalactiae*, Group B *Streptococcus* (GBS) employing on-chip magnetic isolation of GBS based on immiscible filtration assisted by surface tension (IFAST), followed by detection of the isolated GBS using an adenosine triphosphate (ATP) bioluminescence assay. Up to 80% GBS cells were isolated from spiked artificial urine samples with linear responses of bioluminescence signals from isolated cells at 2.3 x 10^2 – 9.1 x 10^5 CFU mL^{-1}, demonstrating great promise for point-of-care detection of pathogenic bacteria in screening urine samples from pregnant women. Practical challenges during initial testing of the developed protocol with urine samples in Kenya are also described.

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Immunomagnetic beads for GBS isolation

Anti-GBS conjugated magnetic beads are not commercially available, and were therefore prepared in-house, employing a non-covalent, and yet stable, streptavidin-biotin bonding.[1] Binding of the commercially available biotinylated antibody against GBS (anti-GBS, Abcam) to GBS cells was first validated utilising a fluorescently-labelled secondary antibody, binding of anti-GBS antibody to GBS cells (Step 1, Fig. S1 and Fig. S2a). By incubating the commercially available streptavidin-labelled magnetic beads (Dynabeads) with biotinylated antibody against GBS (anti-GBS) at room temperature for 30 min, functionalised magnetic beads capable of binding to GBS cells were obtained. The fluorescent beads upon the addition of the same fluorescently-labelled secondary antibody also qualitatively confirmed the streptavidin-biotin bonding (Fig. S2b), demonstrating a successful preparation of anti-GBS conjugated magnetic beads (Step 2, Fig. S1).

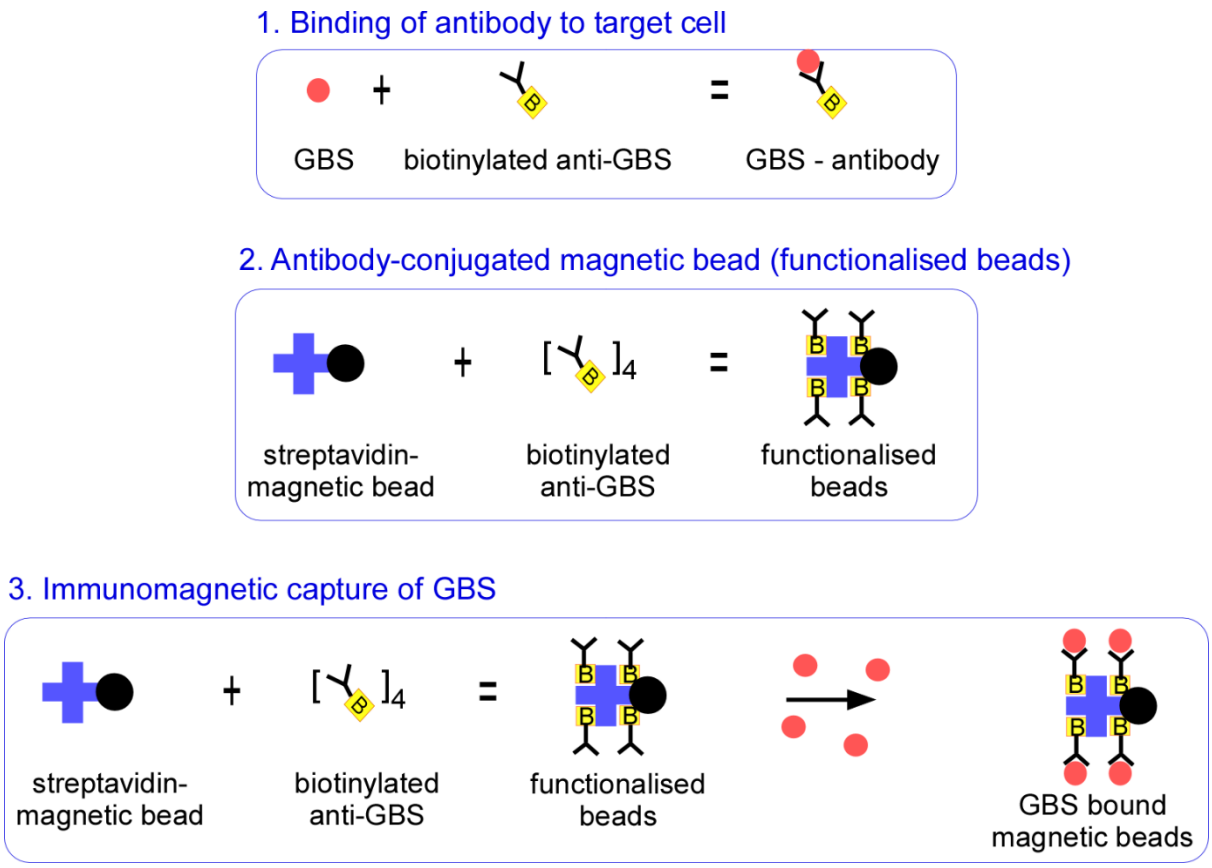

Fig. S1 Immunomagnetic binding of GBS by functionalised magnetic beads.

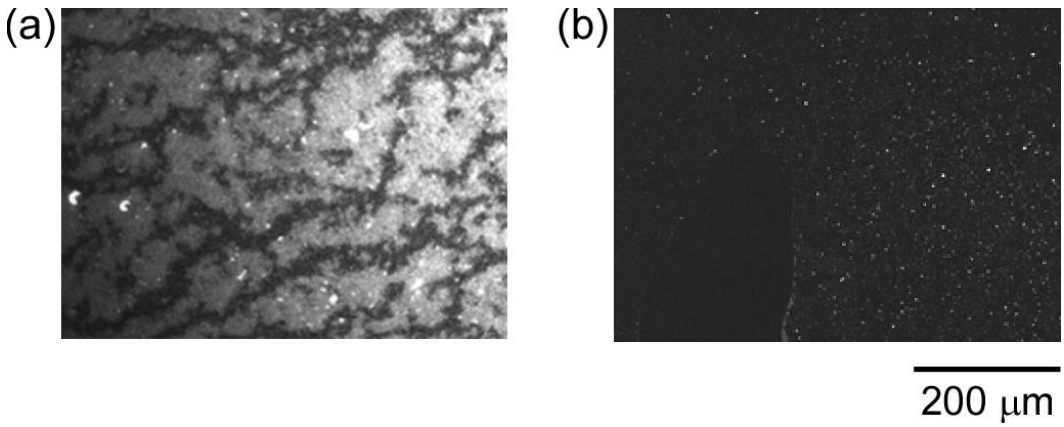

Fig. S2 (a) Fluoresced GBS cells bound to antibody against GBS (Step1, Fig. S1). (b) Fluorescent beads confirmed streptavidin-biotin bonds (Step 2, Fig. S1).

The ability to isolate GBS from spiked buffer (PBS) by the prepared functionalised magnetic beads (step 3, Fig S1) was next quantified. Taking into consideration that testing of the protocol was aimed for a resource-limited laboratory of Kenya, immunomagnetic capture was investigated at room temperature as well as at 4 °C (Fig. S3). Higher capture efficiency was observed at room temperature as a result of accelerated interaction between antibody and GBS cells with increasing temperature. *Ca.* 85% immunomagnetic binding was observed from off-chip tube-based conjugation of anti-GBS antibody to magnetic beads via streptavidin-biotin reaction at room temperature utilising 50 μg antibody per mg beads.

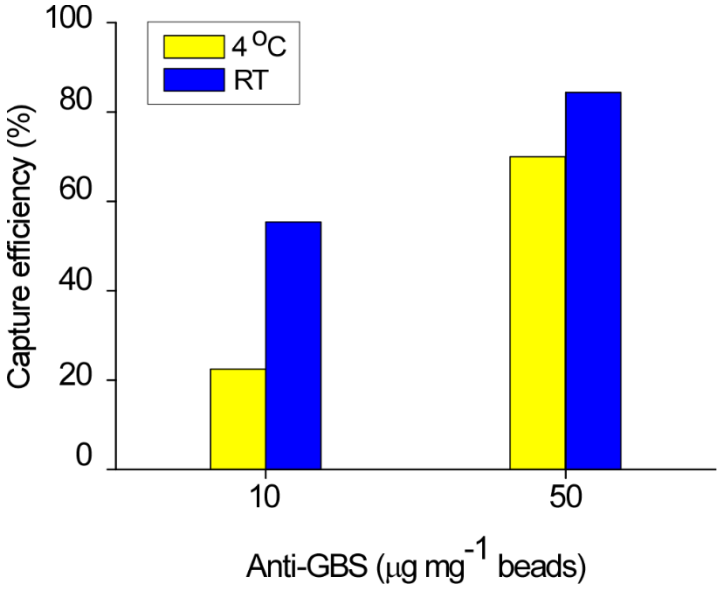

Fig. S3 Off-chip immunomagnetic capture of GBS cells (10^4 CFU mL^{-1}) from spiked phosphate buffer saline samples (n=1).