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A flow cytometry-based assay to determine the ability of anti-*Streptococcus pyogenes* antibodies to mediate monocytic phagocytosis in human sera

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ABSTRACT

Streptococcus pyogenes, commonly referred to as Group A *Streptococcus* (Strep A), causes a spectrum of diseases, with the potential to progress into life-threatening illnesses and autoimmune complications. The escalating threat of antimicrobial resistance, stemming from the prevalent reliance on antibiotic therapies to manage Strep A infections, underscores the critical need for the development of disease control strategies centred around vaccination. Phagocytes play a critical role in controlling Strep A infections, and phagocytosis-replicating assays are essential for vaccine development. Traditionally, such assays have employed whole-blood killing or opsonophagocytic methods using HL-60 cells as neutrophil surrogates. However, assays mimicking Fcγ receptors-phagocytosis in clinical contexts are lacking. Therefore, here we introduce a flow cytometry-based method employing undifferentiated THP-1 cells as monocytic/macrophage model to swiftly evaluate the ability of human sera to induce phagocytosis of Strep A. We extensively characterize the assay's precision, linearity, and quantification limit, ensuring robustness. By testing human pooled serum, the assay proved to be suitable for the comparison of human sera's phagocytic capability against Strep A. This method offers a valuable complementary assay for clinical studies, addressing the gap in assessing FcγR-mediated phagocytosis. By facilitating efficient evaluation of Strep A -phagocyte interactions, it may contribute to elucidating the mechanisms required for the prevention of infections and inform the development of future vaccines and therapeutic advancements against Strep A infections.

1. Introduction

Streptococcus pyogenes, commonly referred to as Group A Streptococcus (Strep A), is a Gram-positive human pathogen responsible for an impressive spectrum of clinical manifestations, ranging from mild, superficial diseases such as pharyngitis and impetigo, to invasive and life-threatening illnesses such as necrotizing fasciitis and toxic shock syndrome (Walker et al., 2014; Brouwer et al., 2023). Recurring Strep A infections are associated with post-infectious diseases, including the development of severe autoimmune sequelae such as acute rheumatic fever (ARF) and rheumatic heart disease (RHD), a condition in which the host raises cross-reactive immune responses against the heart valves, eventually compromising heart function (Carapetis et al., 2016). Strep A infections and autoimmune sequelae are estimated to cause >0.5 million annual deaths worldwide (Vekemans et al., 2019). Despite the extraordinary health impact prophylaxis would have on the health burden caused by Strep A infections (Giannini et al., 2023), there is still no available commercial vaccine (Vekemans et al., 2019; Dale and Walker, 2020; Steer et al., 2016; Sheel et al., 2016). Failure of intervention with first-choice antibiotic penicillin, and the emergence of resistant strains

Abbreviations: ARF, Acute rheumatic fever; EC50, Half Effective Concentration; FcγR, Fc gamma receptor; FITC, Fluorescein isothiocyanate; HL-60, Human Leukemia 60 (cell line); IVIg, Intravenous Immunoglobulin; LoD, Limits of Detection; LoQ, Limit of Quantification; MOI, Multiplicity of infection; OPKA, Opsonophagocytic killing assay; RHD, Rheumatic heart disease; Strep A, Group A Streptococcus; THP-1, (human monocytic cell line).

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to second-choice macrolides are further complicating treatment options (Cattoir, 2022). Considering these challenges, the development of a viable vaccine and alternative approaches is of paramount importance to curb the impact of Strep A infections on global health (Micoli et al., 2021).

Tissue-resident and recruited phagocytes are crucial in controlling the spread of Strep A, as there is evidence that animal models depleted of phagocytic cells as well as neutropenic patients are more susceptible to Strep A infections (Matsumura and Takahashi, 2020). Fittingly, Strep A evolved specialized virulence factors to hinder phagocytosis, limiting recruitment, dampening activity, and interfering with antibody- and complement-mediated uptake (Zinkernagel et al., 2008).

Functional assays replicating phagocytosis in vitro have been indispensable tools for vaccine development and the evaluation of sera's ability to preserve and enhance the host's phagocytic capacity. Phagocytosis activity in the context of Strep A vaccine development was historically evaluated via the whole-blood killing assay originally developed by Todd et al. in 1927 (Todd, 1927) (also referred to as Lancefield assay (Lancefield, 1957), or indirect bactericidal assay), in which whole animal or human blood was used as a source of phagocytic cells (Bensi et al., 2012; Dale et al., 2011; McNeil et al., 2005). To address donor-to-donor variability in cell responses and enhance clinical applicability, a new opsonophagocytic killing assay (OPKA) was introduced. This assay employs differen (Vekemans et al., 2019)tiated HL-60 cells as a substitute for neutrophils and has become the standard method for evaluating the opsonic ability of human sera, suitable for assessing clinical samples (Wagstaffe et al., 2022; Salehi et al., 2018; McGregor et al., 2020; Jones et al., 2018). However, equivalent assays to recapitulate FcγR-mediated phagocytosis in the clinical setting are currently lacking.

THP-1 cells, an immortalized monocyte-derived cell line, play a pivotal role in in vitro research. They are extensively utilized for studying cytokine expression profiles and serve as a starting point for differentiation into various macrophage subtypes. THP-1 cells in their undifferentiated state consistently express phagocytic Fc-gamma receptors (FcγRs), making them an effective model for phagocytic cells (Forrester et al., 2018; Fleit and Kobasiuk, 1991). This feature allows them to be employed as a model system for assessing the internalization of bacterial pathogens, including Strep A (Lindner et al., 2021; Lindner et al., 2020; de Neergaard and Nordenfelt, 2023; Boero et al., 2022).

In this study, we introduce a flow cytometry-based method that offers a swift evaluation of human sera's phagocytic capability against Strep A, leveraging THP-1 cells. We conducted a comprehensive characterization of this approach, encompassing assessments of precision, linearity, and limit of quantification to establish its robustness. This method holds the potential to serve as a valuable complementary assay for use in clinical investigations, either for sero-epidemiological studies or for the evaluation of functional response induced in vaccine trials.

2. Material and methods

2.1. Cell lines, bacterial strains, and sera

THP-1 cells (ATCC, TIB-202) were maintained in a humidified incubator at 37 °C with 5% CO_2 in a sterile cell culture medium composed by RPMI 1640 (Gibco, 52400025) supplemented with 10% FBS not heat-inactivated (Thermofisher Scientific, 10091–148) and 100 units/mL penicillin, 100 μg/mL streptomycin (Thermofisher Scientific; 15140–122). Cells were split three times per week to be maintained at a concentration range between 2×10^5 and 5×10^5 cells/mL.

Strep A strains with different *emm*-types were donated by Prof. Nicole Moreland (University of Auckland): *emm1* (051304), *emm6* (HM2), *emm12* (611025), *emm53* (131465), and *emm75* (GenBank CP033621) (Osowicki et al., 2021), *emm89* (HM66), *emm100* (20123V1I1). The *Δemm* strain was produced internally upon *emm1* gene deletion from the wild-type strain HRO-K-51 kindly provided by the University of Rostock.

Intravenous Immunoglobulin (IVIg) (PRIVIGEN®, Immune Globulin Intravenous (Human), CSL Behring), and three pools of anonymised sera pooled from 30 to 81 individual samples from children aged 24–59 months in The Gambia, were obtained from a study performed for assessing immunological responses to live attenuated vaccine (NCT02972957) (Lindsey et al., 2019). Commercial human IgG-depleted serum (HPLA-SER-GF, Molecular Innovations) was used as a negative matrix to predilute samples or undiluted as negative controls.

2.2. Flow cytometry-based THP-1 opsonophagocytic assay

Strep A strains of interest were grown overnight before the day of the assay. Frozen bacteria were collected from glycerol stocks with a 10 μL loop (Nunc) and inoculated in 5 mL Todd Hewitt Broth (THB) in a 50 mL falcon and incubated at 37 °C static with 5% CO_2, keeping the tube cap loose to allow air exchange. The next morning cultures OD_{600nm} were measured, usually ranging from 0.1 to 0.2. The volume corresponding to 1 OD was transferred to an Eppendorf tube and was pelleted at 10,000 g at room temperature. The pellet corresponding to 1 OD was washed twice in 1 mL carbonate buffer pH 9 (Sigma, C3041) and resuspended in 1 mL of a 0.1 mg/mL solution of FITC in sterile carbonate buffer pH 9. For this purpose, a fluorescein isothiocyanate (FITC) stock solution was prepared by reconstituting 2 mg of FITC powder (Sigma, F7250) in sterile dimethylformamide (DMF) (ThermoFisher, 20673) or any equivalent organic solvent. Bacteria were incubated with FITC for 30 min at room temperature with vigorous shaking and protected from light, to allow the isothiocyanate group in FITC to react with amino-terminal and primary amines on surface proteins. Bacteria were washed twice in phagocytosis buffer composed by RPMI 1640 Medium (Gibco, 5240025) added with 0.5% *w/v* Bovine Serum Albumin (BSA) (Sigma, A7888) and adjusted to OD_{600nm} 0.08, corresponding to a working concentration of 3.75×10^7 bacteria/mL, equivalent to a multiplicity of infection (MOI) of 10 bacteria per cell. Upon testing different ratios, this MOI was confirmed the lowest one to observe on optimal background/fluorescent signal, but maintaining a bacteria to cells ratio that is more similar to the physiological situation in a site of infection.

In parallel, serial dilutions of opsonic agents (IVIg or human pooled sera) were prepared in clear 96-well round bottom sterile plates. Each serum to be tested was serially diluted in phagocytosis buffer in the assay plate (20 μL/well). The starting dilution of each serum in the assay was 2.5× concentrated with respect to the desired final dilution, followed by 3-fold dilution steps up to 7 dilution points. One control well with no serum was included, representing the control for non-antibody-mediated adhesion or uptake, as well as a sample diluted infinite-fold. In the repeatability and linearity results sections IVIg were pre-diluted in IgG-depleted serum to mimic a low titer sample. In the intra-assay repeatability, IVIg were diluted as follows: 1:4 (*emm*1), 1:12 (*emm*12), 1:20 (*emm*53), and 1:3 (*emm*75). Pooled sera were tested from an initial dilution in phagocytosis buffer of 1:30 (Pool 1, Pool 3) or 1:20 (Pool 2).

In the pre-opsonization step 20 μL of bacterial working solution was added to the 20 μL serum dilution in the assay plate. The plate was incubated at 37 °C for 30 min at 750 rpm in a shaking platform inside the CO_2 incubator to minimize stress for bacteria and cells.

In the meantime, undifferentiated THP-1 cells were harvested. Cells were gently mixed with a 25 mL serological pipette and monitored for their concentration and vitality (ideally >95%) via an automated cell counter. The concentration of viable cells was used to calculate the volume of cell culture required to harvest 7.5 million cells per assay plate. Cells were then pelleted at 300 ×g for 5 min at room temperature to discard culture medium antibiotics, the supernatant was discarded by inversion and the cell pellet was resuspended in phagocytosis buffer at the working concentration of 7.5×10^6 cells/mL.

The THP-1 working solution was transferred to a reservoir and 10 μL, corresponding to 75,000 cells, were added to each well of the assay plate

changing tips every row. The assay plates were then incubated for 30 min at 37 °C at 750 rpm on the shaking platform inside the CO_2 5% incubator. Finally, phagocytosis was stopped on ice and by adding 100 µL of BD cytofix (BD, 554655) to each well. The plate was incubated for at least 30 min at 4 °C to inactivate bacteria. The assay plate was spun at 300 ×*g* for 5 min, was then flicked in a basket, and finally, samples were resuspended in 150 µL sterile PBS. Samples were accurately mixed before acquisition to minimize cell adhesion to the well.

2.3. Flow cytometry acquisition and gating strategy

The plate was acquired with BD Accuri C6 Plus flow cytometer with CSampler Plus (Beckton Dickinson & Company) using a pre-set phagocytosis template. A gating was first set in the forward (FSC) and side scatter (SSC) to distinguish THP-1 cells from residual bacteria and debris. The acquisition was set to 5000 cells inside the THP-1 gating, with fast acquisition speed, and a 4500 threshold set for FSC-H.

2.4. Calculations

Samples were analyzed in FlowJo (Beckton Dickinson & Company, version 10.9.0). A gate was placed on THP-1 and the geometric mean of FITC intensity of the whole cell population was extrapolated to minimize manipulation of the operator. GeoMean Fluorescent Intensity (GMFI) values were plotted in Prism (GraphPad, version 9.2.0) and transformed into logarithmic values to fit in a Four Parameter Logistic Regression model for all the sera dilutions tested for each serum; an arbitrary serum dilution of 10^{15} was assigned to the well containing no sera. To allow correct fitting, values beyond the highest point of the curve were deleted. The results of the assay were expressed as the EC50,

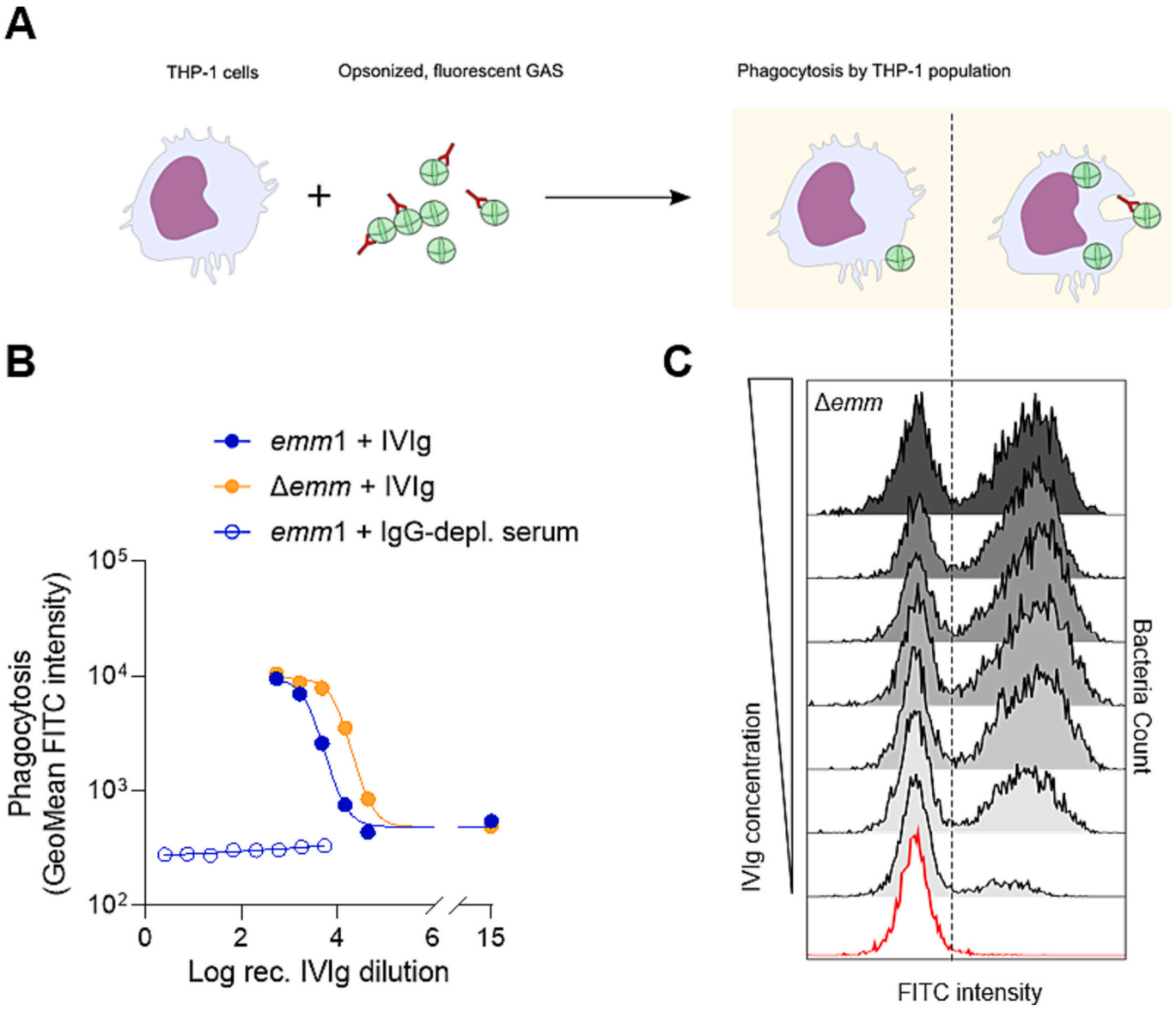

Fig. 1. Overview of the THP-1 phagocytic assay.
A) THP-1 cells exposed to fluorescently labelled bacteria opsonized with progressively diluted IVIg. The extent of bacterial engulfment by THP-1 cells corresponds to IVIg concentration, resulting in varying degrees of cellular fluorescence intensity. **B)** Geometric mean fluorescence intensity extracted from histograms and modelled with a 4PL curve to determine EC50 for each strain-opsonin pairing. The overall THP-1 population's geometric mean values are curve-fitted against opsonin concentrations. **C)** Flow cytometry histograms displaying the distribution of fluorescence intensity within the THP-1 cell population for specific IVIg dilution points utilized during bacteria opsonization. Two discernible populations are evident: non-engulfing cells (corresponding to those incubated with non-opsonized bacteria *in red*) and engulfing cells.

representing the dilution of sera able to mediate 50% of phagocytosis efficiency with respect to the maximal value (opsonic index).

The linear regression analysis was performed with Minitab (Minitab Inc., version 18.1.0,).

The Limit of detection (LoD) and Limit of Quantification (LoQ) of the assay were calculated accordingly to ICH guideline Q2(R1) (Guideline, 2005), applying the following formulas: LoD = 10^(3.3 × SD) × X and LoQ = 10^(10 × SD) × X, where X represents the lowest serum dilution tested in the assay (X = 2.5) and SD the standard deviation of the individual replicates tested.

3. Results

3.1. Principle of the method and assay setup

The phagocytosis assay is designed to assess the FcγR-mediated internalization of specific Strep A strains. The approach is an adjusted version of a flow cytometry-based phagocytosis technique utilized for quantifying *Staphylococcus aureus* uptake by human neutrophils (Boero et al., 2021), subsequently adapted to THP-1 cells (Boero et al., 2022).

In this paper, we set up the method with commercially available IVIg, a known source of opsonizing antibodies derived from the general population (Reglinski et al., 2015). The assay is summarized in Fig. 1. Undifferentiated THP-1 cells are incubated alongside FITC-labelled bacteria that have been pre-opsonized with IVIg or IgG-depleted serum as negative control. The uptake efficiency is assessed for each opsonin source-strain pairing by quantifying the fluorescence augmentation in cells engulfing fluorescent bacteria.

For every IVIg dilution point, the geometric mean fluorescence intensity (GMFI) of the THP-1 cell population is determined and normalized for representation on a 4-parameter curve (4PL) against IVIg dilution. In the context of the phagocytosis assay, it is common to observe a 'hook effect' at higher concentrations, attributed to factors such as the prozone phenomenon and receptor saturation on phagocytic cells' surface (Boero et al., 2021). To ensure accurate fitting, prozone points are omitted from the analysis. To further improve the analysis, we also included in the 4PL fitting the GMFI value of a well with no serum by assigning to it an arbitrary Log dilution of 15 to mimic the GMFI obtained from a serum diluted several billion times, and thus representing the background level of phagocytosis not mediated by antibodies. The use of GMFI detected on the well with no serum, coupled with mathematical fitting of the 4PL regression to all serum data points except the prozone provided solid upper and lower asymptotes of the 4PL curve fitted to data and thus operator-independent analysis of data minimising inter-assay variability. Furthermore, samples with no sera represented internal controls of the assay to confirm the level of non-Fc-mediated phagocytosis. Ultimately, the analysis culminates in extrapolating the half inhibitory concentration (EC50) value, or opsonic index. This value represents the opsonic index and indicates the IVIg concentration needed to mediate bacterial uptake by THP-1 cells at a level representing 50% of the maximum uptake, thus providing a quantification of opsonization efficacy in promoting phagocytosis.

3.2. Repeatability of the assay

The repeatability was evaluated by performing both inter-assay and intra-assay repetitions to evaluate the variability between the opsonic indexes. For inter-assay repetitions five independently handled experiments were performed. The inter-assay CV% ranges from 31 to 46%. The efficiency of phagocytosis is comparable for each of the four strains opsonized with IVIg, which display similar opsonic index (Fig. 2A).

We then tested the intra-assay reproducibility by testing 10 times in a standard assay a sample represented by IVIg pre-diluted in IgG-depleted serum, mimicking a sample with a low but detectable titer, thus representing the conditions with the highest variability to perform the test (Fig. 2B). The intra-assay CV% ranges from 7 to 20%. The standard deviation of the Log transformed opsonic indexes obtained for those tests has been used to calculate the Limit of Detection (LoD) and Quantification (LoQ). LoD and LoQ obtained resulted to be low for all the serotypes (Table 1), therefore the assays are suitable for the detection of low signals, as the IgG-depleted sera tested in the standard assay resulted in no signal.

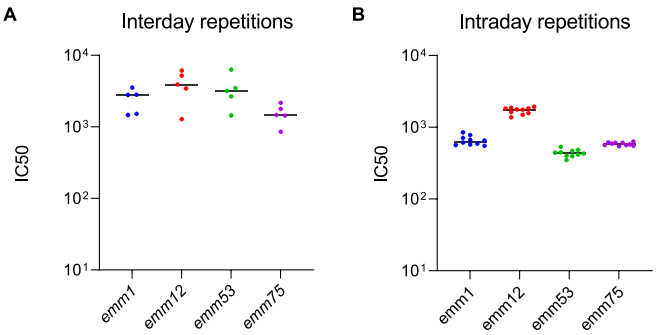

Fig. 2. Repeatability of the assay. **A)** Opsonic indexes of $N = 5$ separate experiments were plotted as single values. Error bars represent the Mean + Standard Error of the Mean. **B)** Opsonic indexes of $N = 10$ intraplate repetitions are plotted as single values. Error bars represent the Mean + Standard Error of the Mean.

3.3. Dilution linearity

To verify the accuracy of the method we measured the dilutional linearity of the assay, verifying the relation between known potency and experimental potency. We constructed target potencies by diluting our reference opsonin source (IVIg) in IgG-depleted serum 1:3, 1:9, and 1:27 times and tested it in our standard assay (Fig. 3A).

Opsonic indexes obtained at the different dilutions were tested in a linear regression model to determine the correlation between the Log-transformed observed values and nominal values, calculated by dividing the observed titer by the dilution factor. We observed a high correlation coefficient ($R^2 > 0.9$) for each strain tested between experimental "observed" opsonic indexes and nominal opsonic indexes (Fig. 3B).

3.4. Testing and comparison of human sera

Flow cytometry-based THP-1 opsonophagocytosis assay is suitable to test and compare the opsonic ability of human sera. We here tested three pools from human sera donated by children exposed to Strep A in endemic countries. These pooled sera were previously characterized in terms of titer of antigen-specific antibodies by our group (Keeley et al., 2022). All samples apart from pool 3, which fails against *emm1* and *emm12* displayed the ability to mediate Fc-mediated opsonophagocytosis against all strains (Fig. 4).

4. Discussion

Assessing the functionality of antibodies generated post-vaccination is crucial to evaluate vaccine efficacy, especially when a correlate of protection is absent. OPKAs are elective methods to gain insights into the functionality of sera, particularly against Gram-positive bacteria

Table 1
Limit of detection and quantification of assays performed with different Strep A strains.

	emm1	*emm12*	*emm53*	*emm75*
LoD [EC50]	4.0	3.6	3.7	3.0
LoQ [EC50]	10.6	7.5	8.2	4.2

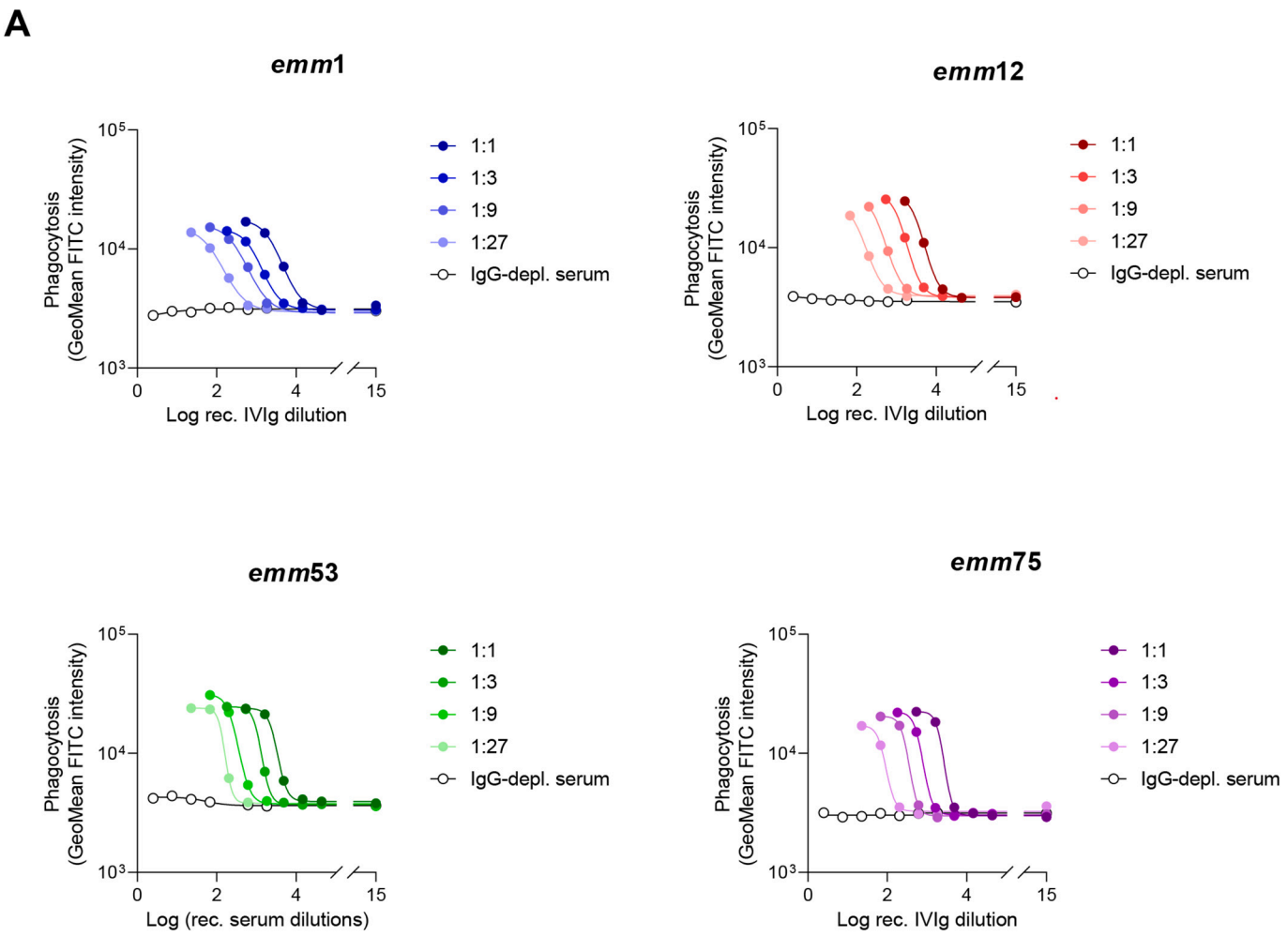

Fig. 3. Linear regression between observed values and nominal values. **A)** Curves of phagocytosis efficiency obtained by serially diluting IVIg in IgG-depleted serum. **B)** Linear regression analysis depicting the relationship between opsonic indexes extrapolated observed values and nominal values. The solid line represents the best-fit linear regression model, while the data points indicate the distribution of observed values across nominal categories. The 95% confidence interval is represented in a dashed line. R2 represents the correlation coefficient.

such as Strep A, which are insensitive to direct complement killing. Such techniques are extensively employed within the field of vaccine development, serving both the validation of vaccines (Wagstaffe et al., 2022; McGregor et al., 2020; Jones et al., 2018) and the exploration of Strep A through numerous investigative studies (Voyich et al., 2004). To circumvent the inherent challenges associated with employing primary human cells, alternative methodologies are preferred that utilize cell lines such as HL-60 and THP-1 since they offer enhanced experimental control and circumvent the variability inherent to primary human cells. Despite the recognized significance of phagocytes in containing Strep A disease, there remains ambiguity regarding the contribution of macrophages versus neutrophils in restraining the progression of Strep A-related diseases. Furthermore, the interplay between Strep A and phagocytes presents a dual aspect: while it holds potential for facilitating immune clearance, it also carries the risk of triggering an excessive and detrimental inflammatory response. This knowledge gap is pivotal to address when designing phagocytic assays intended to serve as surrogates of protective immunity and electing HL-60 as a unique phagocytic model might not be exhaustive. HL-60 cells that are commonly used in traditional OPKA predominantly engage C3b fragments by complement receptors (Fleck et al., 2005). In contrast undifferentiated THP-1 cells do not express substantial levels of complement receptors while they exhibit constant expression of FcγRI (CD64) and FcγRII (CD32), making them a valuable model for studying FcγR-mediated phagocytosis (Forrester et al., 2018; Fleit and Kobasiuk, 1991). FcγRI is a high-affinity receptor for IgG1, IgG3, and IgG4, while FcγRII is a low-affinity receptor for aggregated IgG1, IgG2, and IgG3, subclasses with different effector functions in the vaccine immune response. In combination with the investigation of complement-mediated uptake via classical OPKA, evaluating this facet of phagocytosis could offer a more comprehensive understanding of Strep A clearance, including evasion strategies, such as glycan-hydrolysis and IgG recruitment from their Fc portion, which hinder Fc-effector functions.

The here presented THP-1 OPA demonstrates comparable throughput and automation potential to conventional OPKA assays, while offering distinct advantages such as eliminating the need for THP-1 differentiation to develop phagocytic capacity. While THP-1 are not able to kill their prey, internalization is considered predictive of phagocytic function in other cell types, but these aspects could deserve a systematic comparison. The method can be further optimized by distinguishing internalized and non-internalized bacteria by double staining as conveniently described (de Neergaard and Nordenfelt, 2023). However, it is important to note that the necessity for a flow cytometer and associated software for analysis may pose a logistical challenge, particularly during the transfer of the assay to laboratories with limited

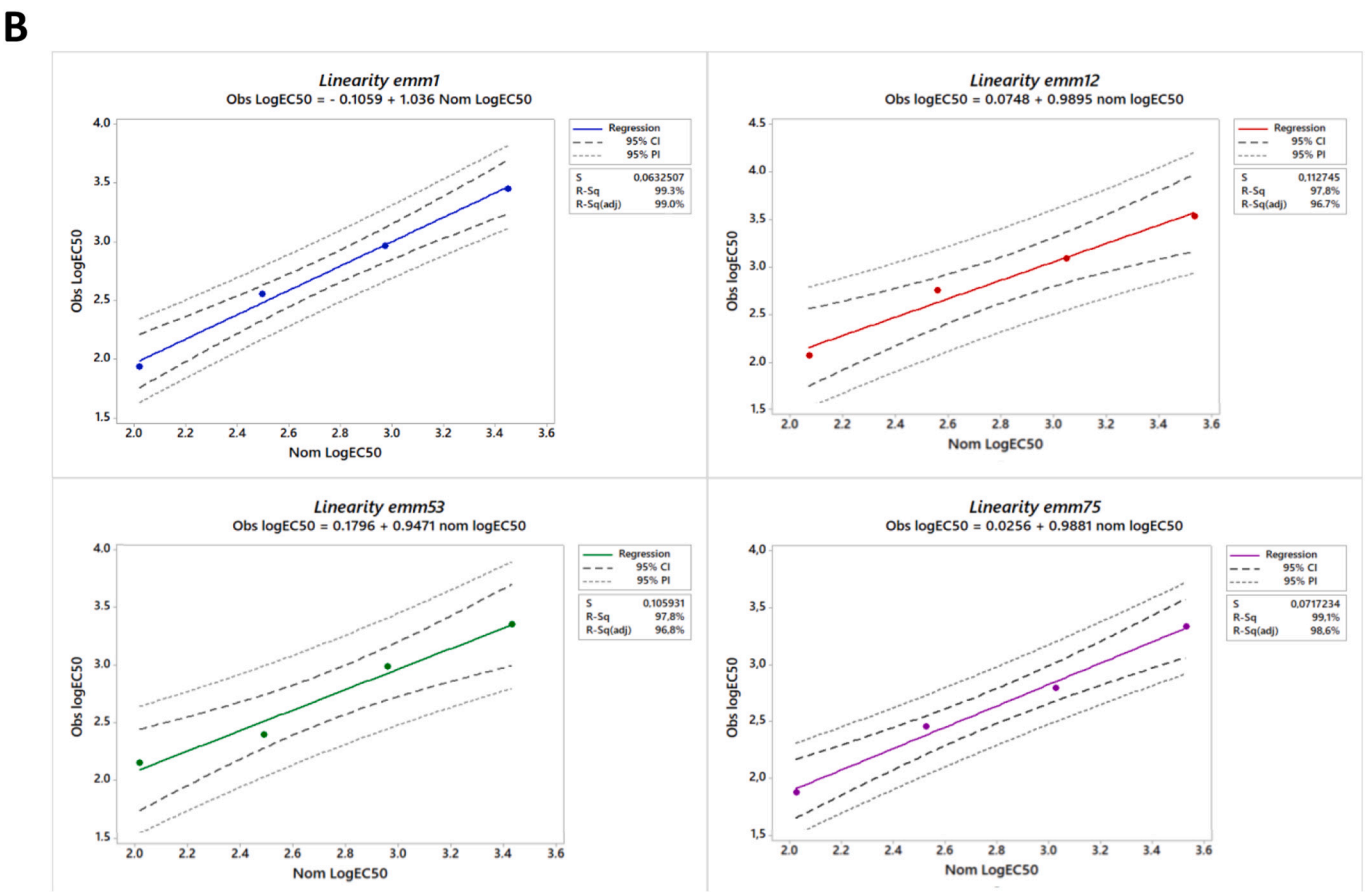

Fig. 3. (*continued*).

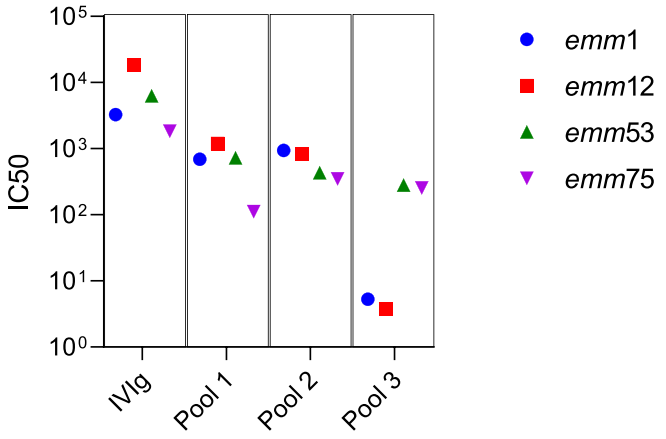

Fig. 4. Opsonic indexes of three pooled sera from exposed children. Opsonic indexes for the phagocytosis of four Strep A strains opsonized with IVIg (positive control) or three pools of human sera from children endemically exposed to Strep A.

resources. Nonetheless, leveraging this technology enables the realization of a same-day assay compared to CFU-based OPKA and costwise is similar since the majority of the costs for both assays are represented by the cell culturing and tips necessary in the assay. Compared to a CFU-based OPKA, THP-1 OPA can be performed during routine laboratory working hours at similar costs. Ultimately, the THP-1 OPA serves as a complementary assay to the traditional OPKA, particularly valuable when HL-60 complement-based assays fail to discern disparities between human sera and offers the possibility to evaluate a different effector function of antibodies.

As OPKA, the THP-1 OPA described here with direct labelling with FITC is applicable across various Strep A strains (seven different *emm* strains and a strain with Emm KO were tested in total, Fig. S1), an aspect especially advantageous for pre-clinical and clinical evaluations, enabling phagocytosis assays on clinical isolates without the need for complex genetic modifications for expression of fluorescent markers. Using IVIg as a standard source of opsonizing antibodies we demonstrated low variability, linearity, and high sensitivity since the assay effectively distinguishes negative and positive opsonin sources even at low antibody titers. Furthermore, to minimize the impact of the inter-assay variability, which is still with CV% acceptable for biological assays according to ICH Guideline (Guideline, 2005), samples collected in different visits of clinical trials can be run within the same session. This capability would enable the measurement of significant fold increase differences, crucial for assessing vaccine responses in clinical trials across multiple visits.

Finally, we successfully demonstrated for the first time that the assay is suitable for comparing the potency of human samples, exemplified by the determination of EC50 values from three pooled sera collected from children exposed to Strep A in endemic regions. In addition to its effective performance with human sera, the study demonstrates its suitability for evaluating opsonophagocytic ability in sera collected after endemic exposure. This insight into the assay's versatility and its capability to assess post-exposure immune responses contributes to an enhanced comprehension of opsonophagocytic dynamics and lays the foundation for potential clinical applications.

5. Conclusions

In summary, we establish and characterize a robust flow cytometry-

based method using THP-1 cells to evaluate the phagocytic response induced by human sera against Strep A. The use of THP-1 cells as an in vitro model of FcγR-mediated phagocytosis will add a complementary tool to clinical serology's armamentarium, which will be crucial in vaccine development to better understand the mechanisms required for protection against this important human pathogen and the further establishment of potential correlates of protection.

5.1. Human biological samples

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki. Use of archived samples for assessing serological responses to Strep A was approved by the joint Gambia Government/Medical Research Council Unit, the Gambia Ethics Committee and the London School of Hygiene and Tropical Medicine Research Ethics Committee (ref. 19163).

Informed Consent Statement: Informed consent was obtained from the parents of all children participating in the study (NCT02972957) from which pooled serum was derived. Parents gave consent for the storage and future usage of the samples for the purposes of scientific research.

CRediT authorship contribution statement

Elena Boero: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Martina Carducci:** Investigation, Writing – review & editing. **Alexander J. Keeley:** Investigation, Resources, Writing – review & editing. **Francesco Berlanda Scorza:** Funding acquisition, Writing – review & editing. **Miren Iturriza:** Funding acquisition, Resources, Writing – review & editing. **Danilo Gomes Moriel:** Conceptualization, Project administration, Resources, Supervision, Writing – review & editing. **Omar Rossi:** Formal analysis, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

This work was undertaken at the request of and sponsored and funded by GlaxoSmithKline Biologicals SA. GSK Vaccines Institute for Global Health Srl is an affiliate of GlaxoSmithKline Biologicals. EB, MC, MI, FBS, MI, DGM, and OR are employees of the GSK group of companies. FBS, DGM, MC, and OR report ownership of GSK shares/share options. AJK is funded by a Wellcome Trust PhD Training Fellowship for Clinicians (225,467/Z/22/Z).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jim.2024.113652.

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