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ORIGINAL RESEARCH

TRANSFUSION

Simulated RBC antibody identification training panels created using SARS-CoV-2 kodecytes and immune plasma

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Abstract

Background: Training is essential to develop and maintain skills required to be a competent serologist, yet samples required to achieve this are often difficult to obtain. We evaluated the feasibility of SARS-CoV-2 peptide modified RBCs (1144-kodecytes) to develop simulated antibody screening and identification panels of reagent RBCs suitable for practical training, recognition, and grading of serologic reactions.

Study Design and Methods: RBCs from a single donor were modified into kodecytes using Kode Technology function-spacer-lipid constructs bearing a short SARS-CoV-2 peptide. Kodecytes and unmodified cells were then arranged in patterns representative of RBC antibody profiles as simulated antibody screening and identification reagent cell panels (SASID), and then tested against immune donor plasma samples containing SARS-CoV-2 antibodies. Manual tube and two different gel card serologic platforms were evaluated by routine techniques. SASID exemplars were created for antibodies including D, C^w, f (ce), Jk^a (strong, weak, dosing), mixtures of D + E, Jk^a + K, Fy^a + E, high and low frequency antibodies and a warm IgG autoantibody.

Results: Kodecytes (positive reactions) and unmodified cells (negative) when arranged and tested in appropriate patterns in SASID panels were able to mimic IgG antibody reactions, and were capable of measuring both accuracy and precision in reaction grading.

Conclusions: Kodecytes can be used to rapidly create in-house simulated yet realistic antibody screening and identification panels suitable for large scale training in the recognition and grading of serologic reactions.

Abbreviations: 1144-kodecytes, kodecytes prepared with FSL-1144; AHG, anti-human globulin (Coombs) reagent; CAT, column agglutination technology; COVID-19, coronavirus disease caused by SARS-CoV-2; DAT, direct antiglobulin (Coombs) test; FSL, function-spacer-lipid Kode Technology constructs also known as Kode constructs; FSL-1144, SARS-CoV-2 function-spacer lipid construct with peptide epitope sequence ELDSFKEELDKYFKN; SASID, simulated RBC Antibody Screening and Identification; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

1 | INTRODUCTION

Training and teaching in university and training laboratories can be hindered by access to quality samples, especially in sufficient quantity to train students in advanced serology. For example, very few teaching facilities would always have available sufficient samples to practically

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teach a class of students the identification of antibodies like anti-Jk^{a}, or more exotic antibodies such as anti-C^{w}. Antibody mixtures, dosage, and antibodies to high-frequency, low-frequency and rare antigens are even more difficult to teach in the practical setting, again due to lack of access to samples. As RBC serology is essentially only the recognition of the reactivity of an antigen on the RBC membrane with an antibody, most serologic laboratory scenarios can be mimicked, provided a suitable antigen–antibody pair can be found. Currently, the majority of individuals globally have antibodies to SARS-CoV-2. We used this phenomenon to evaluate the ability of Kode technology to controllably attach specific amounts of SARS-CoV-2 viral antigen[1–3] onto the RBC membrane and then used immune plasma samples from vaccinated or COVID-19 convalescent individuals to create an antigen–antibody pairing suitable for serologic training requirements.

2 | MATERIALS AND METHODS

2.1 | RBC samples

Washed RBCs obtained from a single group O individual were used unmodified (negatives) and to prepare kodecytes (positive reactions).

2.2 | Kodecytes

Terminology and method for describing FSL constructs and the resultant kodecytes is described in detail elsewhere.[1,4] The SARS-CoV-2 function-spacer lipid construct FSL-1144 (Cat# 801117, GlycoNZ, Auckland, New Zealand) is a 15 amino acid epitope sequence (ELDSFKEELDKYFKN) selected from the conserved region of the SARS-CoV-2 spike protein, based on FSL-1147 previously reported for the determination of SARS-CoV-2 antibodies.[1] FSL-1144 (MW 4144 as a hepta-sodium salt, 0.2 mg/vial) was reconstituted as a 240 μmol/L stock solution with 200 μl of PBS (1 mg/mL) and then aliquoted as 40 μl samples in single-use vials and stored frozen (−20°C) until required. A 2.0 μmol/L working solution was prepared from the thawed (vortexed) 40 μl stock solution and diluted to 4.8 ml (1/120 dilution) in RBC stabilizer solution (ID-CellStab; 005650, Bio-Rad Laboratories, Inc, Hercules, CA, USA), and from the 2.0 μmol/L stock further 1/2 and 1/40 dilutions were prepared as 1.0 and 0.05 μmol/L working reagents. Each 40 μl stock aliquot is able to make at least 900 ml of a 1% suspension of 1.0 μmol/L kodecytes. In brief, the making of kodecytes involved mixing an appropriate volume of the 2.0., 1.0, or 0.05 μmol/L working solution of FSL construct with washed packed RBCs (1:1 v/v), incubating at 37°C for 2 h (with brief mixing at 1 h) and then dilution (1% and 3% suspensions) with storage at 4°C in ID-CellStab. Kodecytes were used within 28 days and FSL working reagents stored at 4°C were used within 2 days.

IgG sensitized kodecytes (direct antiglobulin [Coombs] test; DAT) for use as the autocontrol in the autoantibody simulated panels were created by incubating at least 20 μl of packed 2.0, 1.0, 0.7, 0.05 μmol/L kodecytes with an equal volume of antibody positive plasma for 1 h at 37°C, washing four times and then suspending in RBC stabilizer solution.

2.3 | Plasma samples

Immune plasma samples were obtained from SARS-CoV-2 mRNA vaccinated (V), vaccinated-boosted (B) and COVID-19 convalescent (C) individuals and stored frozen. Samples were screened against 1.0 μmol/L kodecytes in different platforms (Figure 1) to identify those samples most suitable for different simulated scenarios.

2.4 | Serological methodologies

All methods used standard routine serologic techniques. In brief, for tube serology, 50 μl of plasma was mixed with 50 μl of a 3% suspension of kodecytes immediately centrifuged to grade IgM room temperature reactions, then incubated at 37°C for 60 min and graded directly for IgM activity. After washing and addition of anti-human globulin (Epiclone AHG Poly Anti-IgG-C3d, Seqirus, Australia) followed by centrifugation, reactions were graded. The DAT method involved simply mixing the IgG sensitized kodecytes with AHG, centrifugation, and grading.

Two column agglutination technology (CAT) platforms were used and methodologies and scoring systems were as recommended by the manufacturer and as reported elsewhere.[1] The Bio-Rad ID-system used ID-Cards LISS/Coombs (no. 50531, Bio-Rad Laboratories, Inc, Hercules, CA, USA); the Grifols DG Gel system used DG Gel Coombs cards and neutral cards (no. 210342 and 210343, Grifols S.A., Barcelona, Spain). In the DAT method, IgG sensitized kodecytes were added directly to the AHG/Coombs cards, centrifuged and graded.

Digital photographic images of the gel card reactions were imported into Microsoft® PowerPoint, converted into black and white and then a maximum image correction of brightness +40% and contrast +20% was applied.

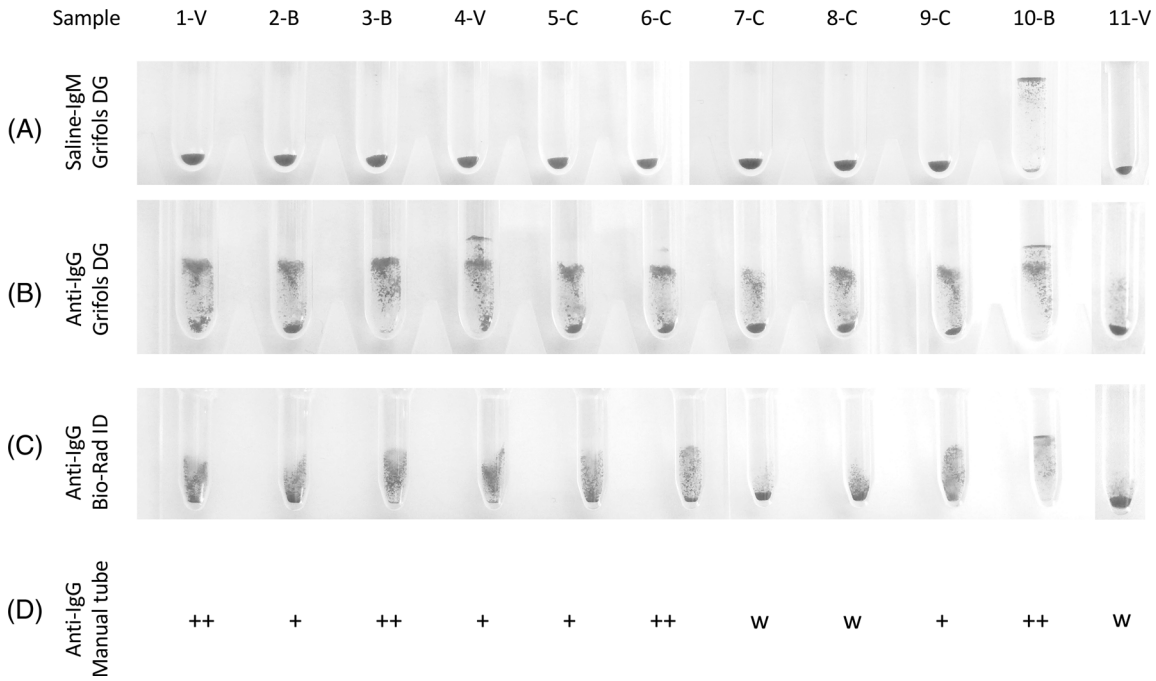

FIGURE 1 Platform sensitivity of 1.0 μmol/L 1144-kodecytes against a range of different vaccinated (V), vaccinated-boosted (B), and convalescent (C) samples. (A) Saline neutral card (IgM) and anti-IgG/AHG (B)–(D). All samples were negative with unmodified cells (not shown). Only sample 10-B had any observable IgM reactivity

2.5 | Simulated antibody screening and identification (SASID) panels

Simulated Antibody Screening and IDentification (SASID) panel worksheets were created (Tables S1 & S2). SASID reagent panels were then constructed by organizing kodecytes (positive reactions) and unmodified RBCs (negatives) to match the desired positive and negative reaction patterns that an antibody would produce according to the SASID panel sheet. The allo-antibody autocontrol was unmodified cells, while the auto-antibody autocontrol sample was kodecytes presensitized with IgG (i.e., DAT positive).

SASID cells were arranged either as a separate three-cell antibody screening panel where at least one of the reagent cells was a kodecyte, or as an 11-cell antibody identification panel. Kodecytes created with FSL at 1.0 μmol/L were primarily used (Figure 1) and expected to give the same positive reaction grades for a given plasma sample (with different grades being observed with different samples). When required, kodecytes 2.0, and 0.05 μmol/L were used to create serologic grade differences in a SASID panel with the same plasma sample (Figures 2D, S6d,e).

Simulated two-stage enzyme panel reactions were created by using samples with IgM activity (e.g., 10-B) and testing direct agglutination in neutral cards against cells suspended in cell preservative solution (albeit with the panel labeled as enzyme modified). The kodecytes in simulated enzyme panels were then organized to represent only enzyme stable antigens (Figure S7).

3 | RESULTS

3.1 | Kodecyte sensitivity

Kodecytes gave a variety of anti-IgG/AHG reactions with different samples when tested against a range of immune plasma samples in the Grifols DG Gel and Bio-Rad ID systems and the manual tube technique (Figure 1). From these results the appropriate plasma samples were selected to give the desired reaction grade against a SASID panel. Changing the strength of individual kodecytes in the panel from 1.0 to 2.0, or 0.05 μmol/L was used to increase or decrease the reaction grade (Figures 2D, S6d,e) for a given plasma sample. There were no observable differences in kodecyte reactions after 28 days storage.

3.2 | Simulated antibody exemplar reactions

Kodecytes and unmodified cells were organized as SASID reagent panel cells (both screening and antibody identification) representing the following exemplar antibody

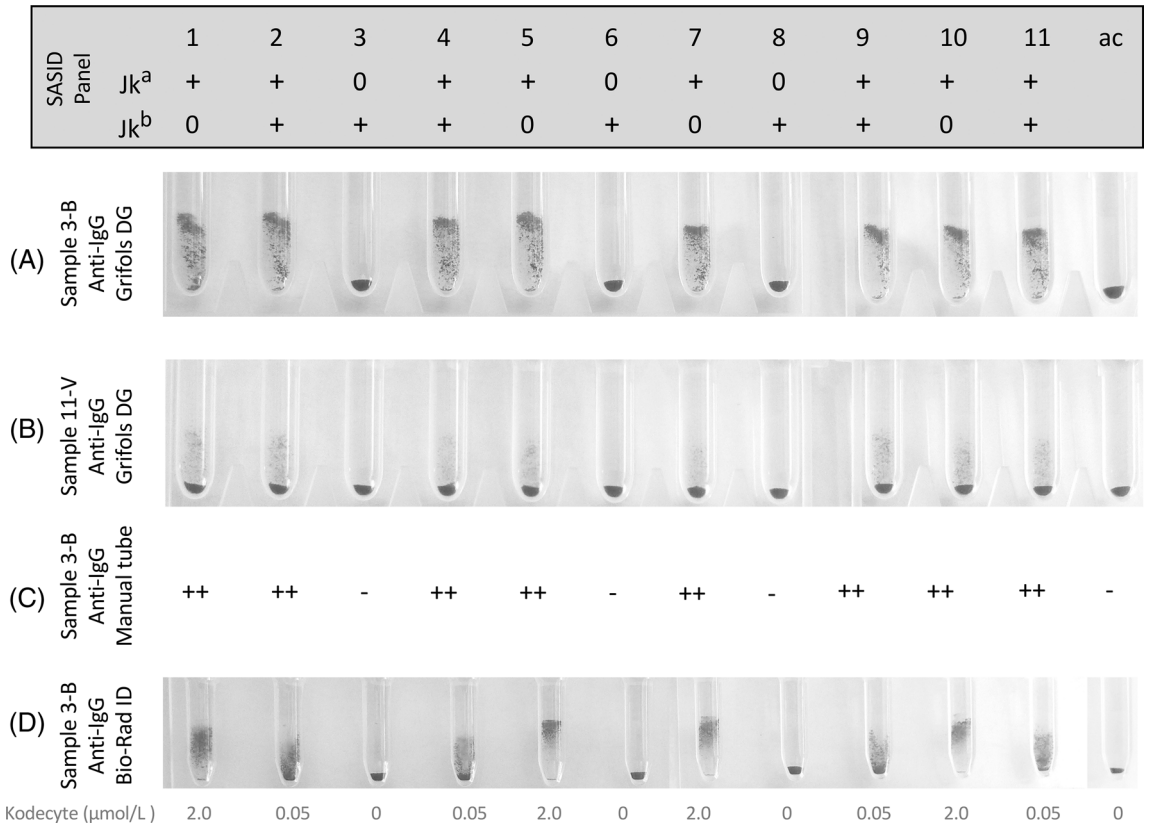

FIGURE 2 Exemplar SASID anti-Jk^a reactions. (A) Simulated strong, (B) simulated weak, (C) manual tube serology grades, and (D) dosage-type reaction grades. Kodecytes in SASID panels (A)–(C) were created with 1.0 µmol/L FSL-1144 while simulated homozygous and heterozygous dosage reactions (D) were created with 2.0 and 0.05 µmol/L kodecytes respectively (as indicated under the gel card columns)

specificities (Tables S1 & S2); Anti-D, –C^w, –f (ce), –Jk^a, and mixtures of –D + E, –Jk^a + K, –Fy^a + E, and antibodies against high (public) and low (private) frequency antigens, and an IgG warm autoantibody.

An exemplar of a SASID panel result of strong, weak, and dosing anti-Jk^a reactions is shown in Figure 2. Exemplars of the other antibody specificities, and reactions in other platforms are shown in Figures S1–S8.

The exemplar of a SASID panel result of a mixture of anti-E and anti-Fy^a (Figure S7) shows the complex combination reaction pattern observed by AHG. Additionally by utilization of a simulated two-stage enzyme treated panel, the underlying anti-E specificity was revealed (due to the apparent destruction of the enzyme sensitive Fy^a antigen). This panel did not use enzyme treated cells, but instead mimicked these reactions by using a saline panel with appropriately organized kodecytes and a plasma sample (10-B) which contained both IgM and IgG anti-SARS-CoV-2.

Exemplars of high (public) and low (private) frequency antigens were created (Figure S8), and the utilization of additional samples of a father and newborn sample (the latter which could also be made DAT positive if desired [Figure S9]) completed a scenario for a low frequency antibody. Distinguishing the high frequency allo-antibody from the IgG-warm auto-antibody was achieved through the autocontrol with an IgG sensitized cell reactive in the antiglobulin panel (Figure S9).

4 | DISCUSSION

The recent development of a diagnostic assay for SARS-CoV-2 antibodies utilizing modified RBCs,[1] and the presence of high quality immune antibodies due to global vaccination programmes and endemic infections, created an antigen–antibody pair suitable to make simulated antibody screening and identification (SASID) panels, based on the previous concept of using unknown antibodies.[5]

The antigen on the RBCs in the SASID panels is not a natural RBC antigen, but is instead a conserved viral peptide antigen sequence of SARS-CoV-2 secondarily attached to the RBC membrane using Kode technology.[1] Secondarily acquired antigens, for example Lewis,[6] Chido/Rodgers,[7] some drugs,[8] and Kode Technology constructs once attached

to the membrane behave like intrinsic RBC antigens and react accordingly when the corresponding antibody is present.[1–3,5–7,9]

Normally reagent cells in an antibody screening or identification panel contain a range of different RBCs selected as a set for their complementary antigenic profiles. However, here with SASID panels, kodecytes and unmodified cells were organized as reagent cell panels (screening and identification) to mimic antigenic variations (see exemplars, Figures 2 & S1–S8).

Simple serologic mimics of antibodies were created by matching positive and negative antigen combination patterns (Figures S4, S6a,b), however, more advanced antibody scenarios often utilized different concentration kodecytes (Figure S6d,e, S7a). Dosage was simulated by using 2.0 and 0.05 μmol/L kodecytes to represent homozygous and heterozygous reactions respectively (Figure 2D). The uncommon antibody against the combination Rh antigen ce (also known as f, and present only on the cells from donors who the carry the c and e genes on the same haplotype[10]) was easily mimicked (Figure S5). An exemplar of a two-stage enzyme panel resolution of an anti-Fy^a + E mixture[11] was also able to be mimicked (Figure S7). Here, the simulated destruction of the Duffy antigen by enzyme treatment was created by using saline panels in neutral cards and a sample (10-B) with IgM anti-SARS-CoV-2 activity (Figure S7d). Alternatively two-stage enzyme panels could be used as it has been previously established that kodecytes can be prepared with two-stage enzyme treated RBCs.[12] It is important to note that the simulated antibody mixture reactions observed in SASID panels are due to a single antigen–antibody reaction and cannot be separated into two or more antibodies (e.g., by absorbtion/elution methods).

Simulated antibodies to low frequency (private) antigens can be achieved by using all unmodified cells (Figure S8a), and providing additional samples of kodecytes to represent samples antigen positive with the low frequency antigens (e.g., the putative father and a newborn blood sample in a hemolytic disease scenario). Similarly this scenario can also be used to mimic drug reactions, where an FSL-1144 solution is provided as the "drug", which when incubated with RBCs will coat them with the "drug", and the resultant kodecytes will then be reactive with autologous immune plasma. The simulation of high frequency antibodies was achieved by using all kodecytes in the panel except the autocontrol (Figure S8b). Likewise by making the entire panel as kodecytes including the autocontrol it will simulate a warm autoantibody (Figure S8c). In this scenario, additionally making the autocontrol IgG positive (DAT positive) can allow for additional DAT testing, and the ability to also make IgG sensitized RBCs (Figure S9) creates the opportunity to also teach direct antiglobulin techniques.

Although many of the exemplars shown could alternatively be achieved by using a natural antibody (e.g., anti-D and using D positive and D negative cells and plasma dilution where necessary) access to large quantities of these samples is generally very limited for training institutions not directly associated with blood services. In contrast SASID panels (positives, negatives, and autocontrols) were made from a single RBC sample and immune plasma samples readily available from the general population.

From a teaching perspective because the actual number of reagent cells in a SASID panel can be restricted to a single positive kodecyte and negative unmodified cell any significant variances observed between the positive reaction grades within a panel is a measure of precision/reproducibility (how close measurements are to each other) and thus provides a useful tool in addressing experimental error. As seen in all examples, reproducibility between kodecytes of the same concentration against the same plasma were almost identical. Additionally because the reagent cells have been created to give a specific reaction grade, accuracy (variance from their true value) can also be measured.

The effort and cost to produce SASID panels is relatively low. Preparation of kodecytes takes less than 2 h, and involves incubating diluted FSL constructs with washed RBCs (no final washing step is required). A single 0.2 mg vial of FSL-1144, which costs approximately US$500, when reconstituted results in five 40 μl stock aliquots. Therefore, a single vial of FSL-1144 is able to make 4.8 liters of 1.0 μmol/L kodecytes as a 1% cell suspension, which equates with a cost for FSL on 2 ml of kodecytes as 21 cents. With respect to additional reagents, regional pricing differences, consumables, and wastage, the final reagent costs for a single 12-cell SASID panel (with 2 ml of each cell and sufficient for at least 30 antibody identifications) is less than US$5. About 12 ml of packed group O RBCs are needed to make forty 12-cell SASID panels and a selection of fresh or frozen plasma samples are also required. As part of the learning experience the antibody positive plasma samples can be found by students screening random samples using a three-cell SASID screening panel (Table S1).

Practical training with realistic samples is essential to develop and maintain skills required to be a competent serologist. SASID panels offer teaching organizations, especially those with limited access to samples, an alternative approach to mimicking antibody specificities. Furthermore, as SASID panels intrinsically measure precision and accuracy in grading serologic reactions, they have the potential to enhance training competencies.

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CONFLICT OF INTEREST

Stephen M Henry is an employee and Stephen M Henry and Holly Perry are stockholders of Kode Biotech, the patent owner of Kode biosurface engineering technology.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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