



COVID-19 antibody screening with SARS-CoV-2 red cell kodecytes using routine serologic diagnostic platforms

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Abstract

Background: The Coronavirus disease 2019 (COVID-19) pandemic is having a major global impact, and the resultant response in the development of new diagnostics is unprecedented. The detection of antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has a role in managing the pandemic. We evaluated the feasibility of using SARS-CoV-2 peptide Kode Technology-modified red cells (C19-kodecytes) to develop an assay compatible with existing routine serologic platforms.

Study Design and Methods: A panel of eight unique red cells modified using Kode Technology function-spacer-lipid constructs and bearing short SARS-CoV-2 peptides was developed (C19-kodecyte assay). Kodecytes were tested against undiluted expected antibody-negative and -positive plasma samples in manual tube and three column agglutination technology (CAT) platforms. Parallel analysis with the same peptides in solid phase by enzyme immunoassays was performed. Evaluation samples included >120 expected negative blood donor samples and >140 COVID-19 convalescent plasma samples, with independent serologic analysis from two centers.

Results: Specificity (negative reaction rate against expected negative samples) in three different CAT platforms against novel C19-kodecytes was >91%, which

Abbreviations: CAT, column agglutination technology; C19-kodecytes, 1147 + 1255-1.5 + 2.5-kodecytes prepared from FSL-1147 (1.5 µmol/L) & FSL-1255 (2.5 µmol/L); EIA, enzyme immunoassay; FSL, function-spacer-lipid Kode Technology construct also known as a Kode construct; N.Z., New Zealand; RUS, Russian Federation; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SCL-SB, Southern Community Laboratories Southern Region SARS-CoV-2 serum-bank

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correlated with published literature. Sensitivity (positive reaction rate against expected positive convalescent, PCR-confirmed samples) ranged from 82% to 97% compared to 77% with the Abbott Architect SARS-CoV-2 IgG assay. Manual tube serology was less sensitive than CAT. Enzyme immunoassay results with some Kode Technology constructs also had high sensitivity.

Conclusions: C19-kodecytes are viable for use as serologic reagent red cells for the detection of SARS-CoV-2 antibody with routine blood antibody screening equipment.

KEYWORDS

Infectious disease testing, intravenous immunoglobulin, kodecyte

The diagnostic detection of antibodies to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus is critically important to address the Coronavirus disease 2019 (COVID-19) pandemic as such assays contribute to the understanding of the susceptibility of a given population^{1,2} and to the preparation of antibody-enriched therapeutic plasma products.^{3,4} Most importantly, SARS-CoV-2 antibody assays may help support public health efforts in distinguishing between natural infection and vaccination rates in populations.

There is a growing number of commercial serological assays that use SARS-CoV-2 Spike (S) and receptor-binding domain protein as practical methods to determine the extent of COVID-19 immunity in a population.^{5–11} Despite this, there is still a need for further assays as viral mutations resulting in new lineages can rapidly arise, and this may affect the robustness of sensitivity and specificity of any specific assay. Similarly, different and evolving vaccination strategies may produce different serologic profiles, with some better suited than others. By having multiple validated assay options available, these risks will be somewhat mitigated, especially if the assay has an intrinsic ability to rapidly adjust its antigenic profile. Likewise, having assays that do not require advanced laboratory instrumentation will be of particular value for developing countries.

Laboratories in most countries are already equipped to undertake routine blood group antibody serology, ranging from simple manual tube serology to fully automated screening. The opportunity, therefore, exists to modify the red cell membrane with SARS-CoV-2 peptides and then use these modified red cells in existing serology platforms.

One such approach for SARS-CoV-2 antibody detection with red cells has been investigated already, where antibodies modified with SARS-CoV-2 peptides have been attached to red cells and used in a serology platform.¹² An alternative is to use the highly adaptable Kode

Technology platform, which utilizes function-spacer-lipid (FSL) constructs to attach epitopes to cells (kodecytes). Kode Technology has successfully been applied to produce a range of carbohydrate blood group antigens, including ABO, Lewis, P, and FORS,^{13,14} and peptide epitopes, such as Miltenberger¹⁵ and cytomegalovirus.¹⁶ Red cell kodecytes have been used for qualitative and quantitative antibody diagnostic purposes.¹⁷

With the peptide sequence of the SARS-CoV-2 virus well established, we investigated the opportunity to create SARS-CoV-2 kodecytes for use on existing routine blood antibody screening diagnostic platforms.

1 | MATERIALS AND METHODS

1.1 | Donor and convalescent plasma samples

Plasma samples from blood donors (July 2020) were obtained from the New Zealand Blood Service (Auckland, New Zealand)—ethics approval AUTEK 20/183. In addition, 77 convalescent plasma samples, predominantly from international repatriations to New Zealand (N.Z.), were collected over the months of June to August 2020 and supplied by the Southern Community Laboratories Southern Region SARS-CoV-2 serum-bank (SCL-SB)—ethics approval HDEC 20/NTB/101. Diagnostics for these SCL-SB samples were performed independently, and all were confirmed as SARS-CoV-2 positive by PCR on at least one occasion, and 59 of 77 were recorded as IgG (nucleocapsid) antibody positive using the Abbott Architect SARS-CoV-2 IgG assay (as reported by SCL-SB). U.S. convalescent plasma samples (July to August 2020) were obtained from 62 donors who had recovered from PCR-confirmed COVID-19 (ClinicalTrials.gov Identifier NCT04360278), and expected negative samples were obtained from 20 blood donors at the Department of Transfusion Medicine, NIH Clinical

Center. Russian convalescent plasma samples were obtained from 14 patients recovered from PCR-confirmed COVID-19 and expected negative samples from eight blood donors.

1.2 | SARS-CoV-2 FSL constructs

Published S protein peptide sequences for SARS-CoV-2 (Genbank QHD43416.1) were used to determine candidate peptide epitopes suitable for construction as FSL constructs.^{13,14,18} Nonglycosylated peptide sequences were selected according to algorithms (Tables S1, S2 and S3), including the use of space-filling models,^{19–21} such as DNASTAR²² of the glycan naked peptide²³ (Figure 1). Eight unique peptide sequences, plus two variations (491H & 888H, with additional histidine tail sequences) were selected (Table 1) and constructed into FSL constructs (Figure 2). Peptides 491 and 888, compatible with FSL construction, were selected from published data.¹²

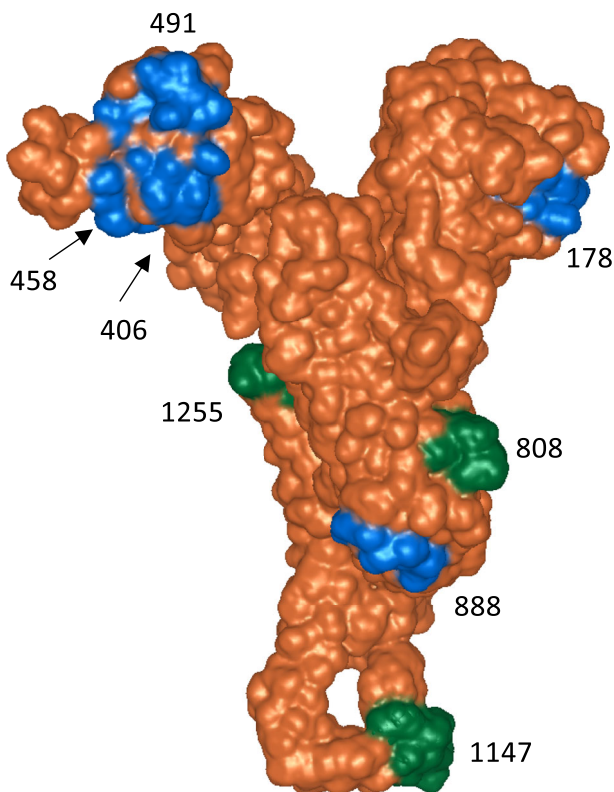


FIGURE 1 Location of the selected peptides sequences in the SARS-CoV-2 spike protein. This space-filling model is shown naked of its significant glycan coating.²³ Numbers refer to the peptide ID in Table 1, and the full length of the peptide epitope is highlighted with color (blue for low and green for high sensitivity and specificity as FSL constructs). Image produced using I-TASSER program for protein three-dimensional structure prediction^{19–21} and DNASTAR²²

1.3 | SARS-CoV-2 kodecytes

Terminology and methodology for describing FSL constructs and the resultant kodecytes are described in detail elsewhere.^{24,25} Essentially, the kodecyte is described by the identification (ID) of the FSL's functional head (Table 1) and the micromolar ($\mu\text{mol/L}$) concentration of the FSL solution used to make it, for example, an 1147-3-kodecyte is a kodecyte made with peptide 1147 at an FSL concentration of 3 $\mu\text{mol/L}$, while an 1147 + 1255-3 + 5-kodecyte is a dual epitope-bearing kodecyte made with a blend of FSL-1147 and FSL-1255 at respective concentrations of 3 and 5 $\mu\text{mol/L}$. The abbreviated term C19-kodecytes was used for the final 1147 + 1255-1.5 + 2.5-kodecyte preparation.

Kodecytes were independently prepared in both Auckland (N.Z.) and Bethesda (U.S.) laboratories using parallel methodology.²⁵ Kodecytes and FSLs were diluted in red cell stabilizer solutions (ID-CellStab 005650, Bio-Rad Laboratories, Inc, Hercules, CA, USA) or Alsever's solution (no. A3551, Sigma-Aldrich, St. Louis, MO, USA).

In brief, the manufacture of kodecytes involves mixing a solution of FSL construct(s) with washed red cells, incubation at 37°C for 2 h, and storage in red cell stabilizer solution at 4°C with no washing required. Kodecytes were rested overnight before use and were used within 21 days.

TABLE 1 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) peptide sequences selected for construction into function-spacer-lipid (FSL) constructs

FSL SARS-CoV-2 peptides		
ID ^a	SARS-CoV-n ^b	Peptide sequence ^c
178	2	DLEGKQGNFKNLREF[C]
406	2	EVRQIAPGQTGKIAD[C]
458	2	KSNLKPFFERDISTEI[C]
491	2	PLQSYGFQPTNGVGY[C]
491H	2	PLQSYGFQPTNGVGY[HHHH][C]
808	2	DPSKPSKRSFIEDLL[C]
888	2	FGAGAALQIPFAMQM[C]
888H	2	FGAGAALQIPFAMQM[HHH][C]
1147	1,2	[C]SFKEELDKYFKNHTS
1255	1,2	[C]KFDEDDSEPVLKGVK

^aID is based on the initial amino acid in the SARS-CoV-2 consensus sequence and includes an H if the sequence has an additional histidine tail sequence appended.

^bSARS-CoV-n indicates if specific to SARS-CoV-2 (2) or common to both SARS-CoV-1 and SARS-CoV-2 (1,2).

^cSARS-CoV-2 peptide sequence (relating to the ID number) together with additional residues not part of the natural peptide sequence, including the conjugation cysteine [C] and solubilization histidine [H] residues. The location of the [C] cysteine residue (used to conjugate the peptide to the spacer) also indicates the region of peptide closest to the cell membrane.

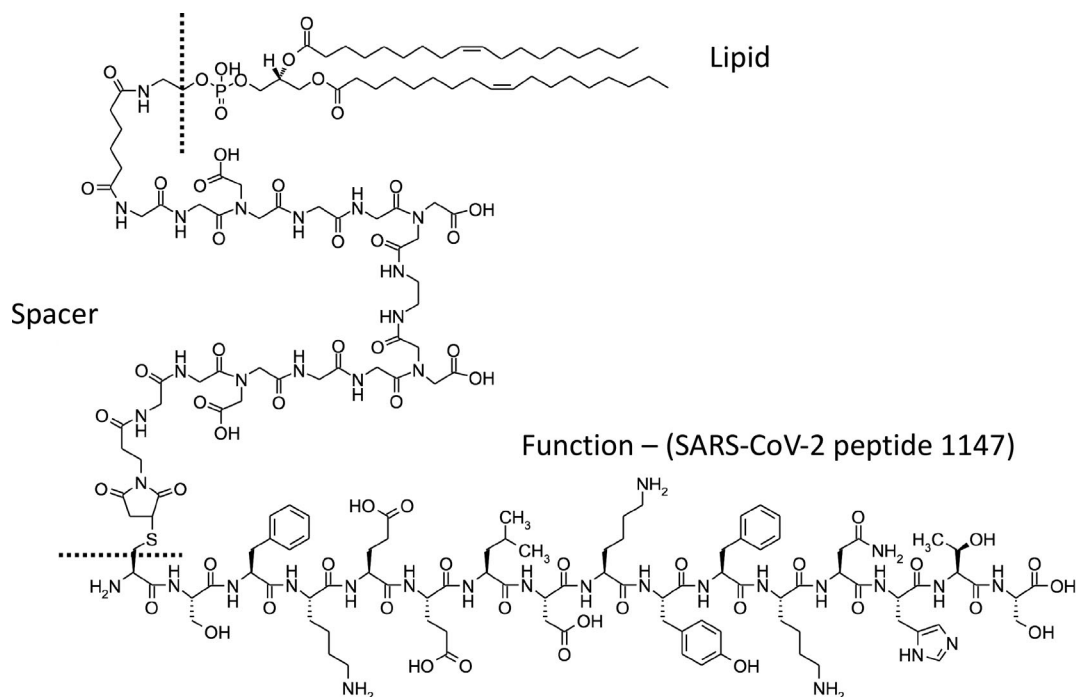


FIGURE 2 Representative schematic diagram a function-spacer-lipid (FSL) construct (FSL-1147). The FSL construct consists of a lipid phosphate moiety (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine) conjugated to the spacer (carboxymethylglycine), which is conjugated via a cysteine SH group to the variable peptide functional head (in this example, the peptide is ID 1147, Table 1)

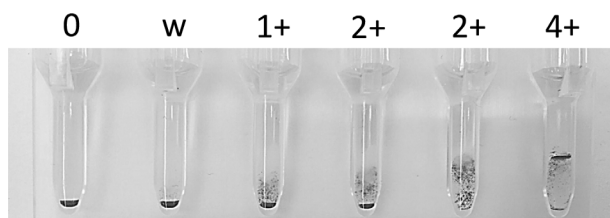


FIGURE 3 Typical agglutination reactions observed with C19-kodeocytes in column agglutination technology. Shown are results of healthy donor sample (negative control) and 5 convalescent COVID-19 recovered plasma donor samples in the ID-micro typing system, anti-IgG card. Serologic grades assigned to the reactions are indicated above the microwells. Positive samples were selected and arranged as examples of observed increasing reactivity. Related images for Bio-Rad ID-system and the Grifols DG Gel system are shown in Figure S1

1.4 | SARS-CoV-2 kodecyte assay

For tube serology, 50 μ l of plasma was mixed with 40 μ l of a 5% suspension of kodeocytes immediately centrifuged to grade IgM room temperature (IgM-RT) reactions, then incubated at 37°C for 60 min, and graded directly (IgM-37). After washing and addition of anti-human globin (Epiclone AHG Poly Anti-IgG-C3d, Seqirus, Australia), antiglobulin reactions were graded (AHG). Three CAT platforms were used, and methodologies and scoring systems were as recommended by the manufacturer,

including the use of the grade “w” to indicate weak positive reaction (Figure 3). 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); and the ID-Micro Typing System used polyclonal rabbit anti-human IgG cards (no. MTS084024; Ortho Clinical Diagnostics, Raritan, NJ, USA). All samples reactive to SARS-CoV-2 kodeocytes were also tested against unmodified and unrelated FSL control cells.

1.5 | Solid-phase enzyme immunoassays with peptides

Eight peptides (178, 406, 458, 808, 888, 888H, 1147, 1255) used to make FSL constructs for the preparation of kodeocytes (Table 1) were also tested by in-house enzyme immunoassay (EIA). Two different approaches were used to immobilize the peptide epitopes in 96-well microtiter plates. The first approach (polyacrylamide [PAA]-EIA) used peptides conjugated to PAA, which were used to coat the microplates (described in detail elsewhere).²⁶ The second proof-of-concept approach (FSL-EIA) used FSL constructs 1147 & 1255 and attached these to 96-well microplates (Nunc Maxisorp, Sigma-Aldrich, St Louis, MO, USA) precoated with lipid. In brief, 100 μ l of ethanol-

TABLE 2 Initial specificity and sensitivity analysis of all severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) kodecytes

Kodecyte ID	μM^b	n (N.Z. + United States [U.S.]) ^c	Specificity (expected negative)					Sensitivity (expected positive)		
			Tube serology (New Zealand [N.Z.])			Column agglutination technology (CAT) ^a		CAT		
			IgM-RT	IgM-37	AHG	nPos (N.Z. + U.S.) ^c	% Pos	n (US)	n Pos	% Pos
178	10	68 + 20	1	0	0	1 + 0	1%	62	1	2%
406	10	72 + 20	1	0	2			6	0	0%
458	10	60 + 20	3	0	0	2 + 1	4%	62	5	8%
491	10	72 + 20	3	0	0			6	0	0%
491H	10	72 + 20	4	0	2			6	0	0%
808	10	100 + 20	0	0	0	3 + 3	5%	62	28	45%
888	10	72 + 20	2	0	0			6	0	0%
888H	10	72 + 20	1	0	0			6	0	0%
1147	3	100 + 20	0	0	2	5 + 2	6%	62	48	77%
1255	5	100 + 20	3	0	1	1 + 0	1%	62	32	52%
Untreated	0	^d	0	0	0	0		62	0	0%

^aCAT results are split into N.Z. and U.S. results representing Bio-Rad and MTS platforms, respectively. nPos indicates the number of positive reactions by N.Z. and U.S. laboratory, while % Pos uses combined CAT data to calculate percentage positive rate. Gaps in the table indicate analysis not done.

^b μM refers to the $\mu\text{mol/L}$ concentration of function-spacer-lipid used to make the kodecyte. Results are only shown for the single concentration considered most appropriate for diagnostic use.

^cThe number of samples is divided into N.Z. and U.S. samples (separated by the + symbol).

^dAll samples reactive as positives were also tested and found negative against unmodified cells used to make kodecytes.

containing lecithin (25 $\mu\text{g/ml}$) and cholesterol (50 $\mu\text{g/ml}$) were added to the wells of the plate and dried at 37°C for 60 min and then left at RT overnight until completely dry. In the next stage, FSL peptides (10 $\mu\text{g/ml}$) in 100 μl of buffer were then added to the plate wells and incubated for 60 min at 47°C. After peptide coating the microplates, both PAA-EIA and FSL-EIA methods were standard procedures with secondary labeled antibodies and color development read using a microplate spectrophotometer.²⁶

2 | RESULTS

Two terms are used to describe diagnostic accuracy in this article according to FDA criteria.²⁷ Specificity is the estimated proportion of subjects without the target condition in whom the test is negative. Sensitivity is the estimated proportion of subjects with the target condition in whom the test is positive.

2.1 | Preliminary screening kodecyte specificity and sensitivity

The initial method development in manual and CAT platforms involved evaluating each FSL construct for specificity as kodecytes over the range of 3–20 $\mu\text{mol/L}$

against expected negative samples. Concentrations of kodecytes were then evaluated for sensitivity against convalescent samples to select those kodecytes that showed appropriate specificity and sensitivity. The sensitivity and specificity for the single concentration considered the most appropriate for diagnostic use for all kodecytes evaluated are summarized in Table 2. Only kodecytes made with FSLs 808, 1147, and 1255 showed adequate sensitivity. However, the average reaction strength of 808-kodecytes was half that of 1255-kodecytes and gave no additional information over 1147-kodecytes and so were not tested further. From these initial experiments, the two most promising FSL candidates (1147 and 1255) were selected for extended analysis along with a dual-epitope kodecyte made with a blend of FSLs 1147 and 1255.

2.2 | Tuning kodecyte specificity and sensitivity: 1147 and 1255

Kodecytes were prepared with FSLs 1147 and 1255 at two different micromolar concentrations (3 + 5 $\mu\text{mol/L}$ and “half strength” 1.5 + 2.5 $\mu\text{mol/L}$), both singly and as dual 1147 + 1255-kodecytes. A comparison of the results obtained with the 1147 + 1255–3 + 5 kodecytes and half-strength 1147 + 1255–1.5 + 2.5 kodecytes against the 56

TABLE 3 C19-kodecytes in three different column agglutination technology (CAT) platforms and the Abbott Architect enzyme immunoassay (EIA) IgG antibody results against PCR SARS-CoV-2-positive samples

COVID-19 Status	Antibody positive results in Coronavirus Disease 2019 (COVID-19) PCR-positive samples							
	CAT (anti-IgG) C19-kodecyte ^a assay							EIA ^b
	MTS (U.S.)		Bio-Rad (N.Z.)		Grifols (N.Z.)			
PCR Positive	49/54	91%	63/77 ^c	82%	75/77 ^c	97%	59/77 ^c	77%
Expected Negative ^d	0/19	100%	4/100	4%	9/100	9%		

^a1147 + 1255-3 + 5-kodecytes were also tested but did not differ (not shown).

^bEIA – Abbott Architect SARS-CoV-2 IgG enzyme immunoassay results as reported by the Southern Community Laboratories Southern Region SARS-CoV-2 serum bank (SCL-SB).

^c77 PCR-confirmed SARS-CoV-2-positive samples supplied by SCL-SB; 7% of these samples also had C19-kodecyte IgM saline CAT activity.

^dExpected negative samples are blood donor samples.

TABLE 4 C19-kodecyte tube serology results against PCR severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-positive samples

Coronavirus Disease 2019 (COVID-19) status	Tube serology C19-kodecyte assay					
	IgM-RT		IgM-37		AHG (anti-IgG)	
PCR Positive	0/77	0%	0/77	0%	51/77	66%
Expected Negative	1/100	1%	1/100	1%	2/100	2%

U.S. convalescent samples showed a slight reduction in score for five samples and no change in the number of positive samples. However, in the 20 negative samples, specificity improved with epitope dilution to 1.5 + 2.5 µmol/L. In addition, plasma samples diluted to 1:3 in PBS were evaluated; however, dilution did not improve specificity and instead substantially reduced sensitivity (not shown).

On balance, it was considered that the performance of the 1147 + 1255-1.5 + 2.5 kodecytes (hereinafter called C19-kodecytes) against undiluted plasma (Figure 3) was the best generic formulation for further evaluation.

2.3 | Evaluation of C19-kodecytes

Extended sensitivity analysis of the C19-kodecytes was undertaken with the SCL-SB samples (Abbott Architect SARS-CoV-2 IgG known status) and NIH convalescent donors who had recovered from PCR-confirmed COVID-19. The Grifols DG system reacted with 97%, Ortho MTS system with 91%, and the Bio-Rad ID system with 82% of these PCR-confirmed SARS-CoV-2 positive samples, compared with 77% by EIA (Table 3). It should also be noted

that the specificity rate was also different, with the Grifols DG system reacting with 9% and Bio-Rad platform with 4% of expected negative samples. The specificity rate for the EIA assay was not reported by SCL-SB.

Further analysis of the differences between the Grifols DG, Bio-Rad-ID, and Abbott Architect systems (Figure S2 and Table S4) revealed that C19-kodecytes in the Grifols DG platform obtained 2+ or greater grades in 69 of 77 (90%) of samples, while the Bio-Rad-ID platform scored 2+ or greater grades in only 24% of samples (and 18% of these expected positive samples were negative).

To establish the contribution of IgM to the results observed in antiglobulin CAT cards, the SCL-SB samples were also tested in neutral CAT cards. Of the 73 available anti-IgG CAT-positive samples, 5 (7%) were also positive in the neutral cards, indicating the detection of IgM activity. In all examples, the anti-IgG card grades were at least one grade stronger than in neutral cards (results not shown), indicating the copresence of IgG. None of these IgM-reactive samples reacted with unmodified cells, nor were there any positive samples at IgM-RT or IgM-37 by manual serology with C19-kodecytes (Table 4).

Although manual tube serology showed 66% sensitivity and 98% specificity for IgG (Table 4), reactions were much weaker than those observed in CAT. Of the

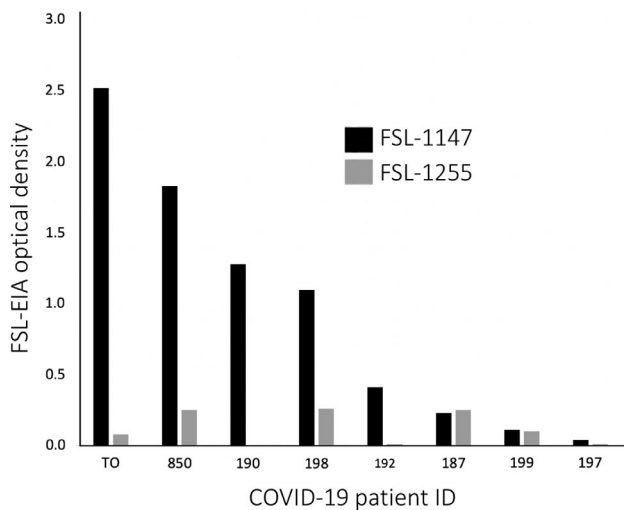


FIGURE 4 Function-spacer-lipid (FSL)-enzyme immunoassay (EIA) of convalescent plasma samples. Eight Coronavirus Disease 2019 (COVID-19) convalescent plasma Russian Federation samples diluted 1:3 and tested against FSL-1147 and FSL-1255 by EIA. Patients 850,190, and 187 had severe COVID-19, while the remainder were mild or asymptomatic. There was insufficient sample 190 to test against FSL-1255

manual tube serology-positive results recorded for AHG, 25 of 51 (49%) were w reactions, and 44 of 51 (86%) were either w or 1+. Only 7 of 51(14%) reactions were grade 2 +, and none were grade 3+ or 4+. Doubling the strength of the kodecyte formulation to 1147 + 1255–3 + 5-kodecytes did not improve the rate of sensitivity (not shown).

2.4 | Specificity and Sensitivity: Solid-phase EIA assays

The in-house PAA-EIA assay IgG/IgM results against PAA-conjugated peptides for Russian Federation (RUS) blood donors and convalescent plasma samples did not show clear specificity or sensitivity, although the results supported the use of peptides 1147 and 1255 (not shown).

The in-house proof-of-concept FSL-EIA assay with solid-phase FSL-1147 and 1:3 diluted plasma was clearly able to identify SARS-CoV-2 antibody in five of eight COVID-19 convalescent samples (Figure 4). In contrast, FSL-1255 did not show sensitivity.

3 | DISCUSSION

Plasma as a source of therapeutic antibodies is still considered a useful therapeutic approach,^{28,29} and in early clinical trials with SARS-CoV-2 antibody-enriched

intravenous immunoglobulin, improved clinical outcomes were observed.^{3,4} Demand for these products will require mass screening of blood donors.

Kode Technology is a simple technique that attaches small molecules onto the outside of the red cell and then uses these modified red cells (kodecytes) in existing serologic diagnostic platforms.^{13–16} Other than kodecyte reagent red cells, the kodecyte assay methodology that we have developed in this study is identical to that used for routine antiglobulin screening for red cell antibodies. Because red cell antibody detection by manual tube or CAT is present in almost every transfusion laboratory, the kodecyte assay can be easily used by almost any serologic laboratory, including those in most developing economies. We therefore considered the possibility that SARS-CoV-2 kodecytes could also be created using established kodecyte methods^{14,15} with a view that these kodecytes would also be of value to blood services that could undertake mass population sample screening using surplus laboratory capacity.

The design approach taken to develop this SARS-CoV-2 kodecyte antibody diagnostic was to first identify potential nonglycosylated epitopes on the S protein using a range of predictive algorithms and online tools (Tables S1 and S2). Although Kode Technology is able to attach glycans onto cells,^{13,14,16,17} the SARS-CoV-2 kodecyte assay was restricted to nonglycosylated peptide epitopes, both because of ease of manufacture and no diagnostic glycan antibody signatures for COVID-19 are as yet recognized. The selected peptides were then further refined to be compatible with FSL conjugation chemistry,¹⁸ and eight candidates were chosen for construction as Kode FSL constructs (Table 1). These FSL constructs were then used to create kodecytes at different concentrations, and they were tested against expected negative (specificity) and positive (sensitivity) samples in manual and CAT platforms to find useable concentration ranges that gave acceptable specificity and sensitivity. This range was found to be 5 $\mu\text{mol/L}$ or less. From the initial eight candidates, three FSL constructs (808, 1147, and 1255) showed the most promising sensitivity, which was further reduced to two after FSL-808 was sidelined. The two constructs FSL-1147 and 1255 were then formulated as single and dual epitope-bearing kodecytes for further analyses. We found that better sensitivity and specificity was achieved with 1147 + 1255–1.5 + 2.5-kodecytes, which were renamed C19-kodecytes. It is important to note this was a proof-of-principle trial and final optimizations for the different kodecyte formulations on different platforms have not yet been performed, and as expected, differences between platforms were observed (Table 3). We expected to achieve better specificity on the Grifols platform and improved sensitivity on the Bio-Rad and manual tube platforms than was observed with the current base formulation of the

C19-kodeocytes described in this study. We are currently undertaking these extensive optimizations in preparation for clinical studies with a prototype assay, including determining extended stability and storage parameters.

Evaluating the performance of C19-kodeocytes against convalescent plasma found that the three different CAT platforms were all able to detect antibodies in the majority of samples from COVID-19 convalescent patients (Table 3). The results observed were almost certainly due to IgG antibodies, and although IgM will contribute to the reactions in CAT anti-IgG cards, saline reactions indicated a low-level contribution (about 7%) of IgM. This was expected as, in previous studies with Miltenbeger kodeocytes, it was found that IgM is poorly reactive with kodeocytes made with peptide-FSLs.^{14,15} Overall, the C19-kodeocyte assay detected more antibody-positive samples than were detected with the Abbott Architect SARS-CoV-2 IgG enzyme immunoassay. However, it should be appreciated that alternative and next-generation EIA platforms would be expected to have improved sensitivity and specificity. Extensive comparative analysis of the CAT and EIA platforms, including a scatterplot analysis (Figure S2), did not reveal any clear reason for differences in performance. However, it must be appreciated that, together with the significant fundamental differences in assay methodology, the EIA assay uses diluted plasma, while the kodeocyte assay uses undiluted plasma. It is reasonable to expect that lower levels of antibody will be easier to detect in undiluted plasma (although the level of epitope on the kodeocyte may offset this somewhat).¹⁴ When 1:3 dilutions of plasma were evaluated against kodeocytes, there was a substantial loss in positive reactivity, supporting the loss of sensitivity caused by dilution.

Parallel to the sensitivity for diagnostic assays is specificity, where an undesired number of false-positive reactions will invalidate the usefulness of an assay. It is reported that antibodies to the coronavirus S protein (from endemic common corona viruses) are expected in up to about 10% of the general population.³⁰⁻³² Consequently, a nonspecificity rate of 3%–5% reactivity with samples that have not had contact with SARS-CoV-2 was considered acceptable. The C19-kodeocytes were found to react with up to 9% of expected negative samples in the Grifols DG system (which showed the highest degree of sensitivity at 97%); however, the nonspecificity rate was a more acceptable 4% with the Bio-Rad platform, but the sensitivity rate was lower at 81%. The MTS platform appeared to have a performance in-between these two platforms, with a sensitivity of 91%, and further testing is required to more accurately define specificity. Manual tube serology was less sensitive than CAT and only reacted with two-thirds of the convalescent plasma

samples and with generally w reaction grades. Further optimization of the assay for manual serological use is required for the detection of antibody; however, the assay as is would be suitable for the detection of samples with higher levels of antibody (especially as the kodeocyte assay is semiquantitative).¹⁷

In addition to the kodeocyte assay, two solid-phase assays using the same peptides as those used to make the FSL constructs were designed to evaluate the usefulness of these peptides by solid phase. These two solid-phase EIA included one where the peptide was conjugated to PAA and immobilized on a microplate (PAA-EIA), and the other used the same FSL constructs that were used to make kodeocytes but instead immobilized them in a solid-phase lipid layer onto microplates. The PAA-EIA did not show sensitivity or specificity in contrast to the FSL-EIA, which demonstrated a viable proof of concept and could also be used with undiluted plasma (results not shown), but further analysis of a larger dataset is still needed.

Intriguingly, the FSL-EIA outperformed the PAA-EIA assay, suggesting that the presentation of small peptide epitopes on FSL constructs is a favorable form of presentation. Furthermore, the presentation of the FSL in the red cell membrane may be more favorable (than in EIA) as it allows for the use of undiluted plasma. It can be speculated that the glycocalyx of the red cell may be able to buffer nonspecificity from low-affinity cross-reactive antibodies (an effect managed by plasma dilution in EIA assays).

The manufacture of kodeocytes is a very simple process,^{13,25} requiring only the contacting of a solution of FSL construct(s) dispersed in buffered saline with washed red cells, incubation at 37°C for 2 h, and then dispensing them “ready for use” (washing is not required). The prepared kodeocytes are then stored and used as normal reagent red cells. One mg of a 15-amino acid peptide FSL construct will make about 70 ml of 5 μ mol/L kodeocytes (packed cells). If these kodeocytes are then diluted to 1% for CAT technology, this will result in 7 L of reagent red cells, and if 50 μ l is used per assay, then 1 mg will enable more than 100,000 CAT assays. Although RBCs were used to prepare kodeocytes in this study, FSL constructs can also be attached to noncellular surfaces,^{24,33} including microspheres and lateral flow membranes or immobilized cell membranes, opening up further possibilities to develop novel diagnostics.

Overall, the kodeocyte assay was able to achieve specificity and sensitivity at least equivalent to an established EIA antibody diagnostic. Due to the cassette design of Kode Technology, it is highly adaptable,^{13-18,24-26} and changing the antigenic epitope on a Kode FSL construct can be achieved within a few weeks, allowing for rapid response to new strains arising with novel antigenic

mutations. Other than determining optimal concentrations for sensitivity and specificity, no other modifications to the methodology for use are required.

This article describes an adaptable platform technology able to be easily accommodated into almost all existing transfusion diagnostic laboratories, including those with limited infrastructure, and will allow for this sector to actively participate in the screening for SARS-CoV-2 antibodies, both for population needs and therapeutic uses.

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STATEMENT OF DISCLAIMER

The views expressed do not necessarily represent the view of the National Institutes of Health, the Department of Health and Human Services, or the U.S. Federal Government.

CONFLICT OF INTEREST

SMH, NVB, and ECW are employees and stockholders of Kode Biotech, the patent owner of Kode biosurface engineering technology. All other authors report no conflict of interest.

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SUPPORTING INFORMATION

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

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