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Purification of total IgG from SARS-CoV-2 convalescent serum

Arfat Lusinanto[1*], Ria Syafitri Evi Gantini[2], Dwirini Retno Gunarti[3], Mohamad Sadikin[3]

[1]Master's Programme in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[2]Akademi Bakti Kemanusiaan, Indonesian Red Cross, Jakarta, Indonesia
[3]Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[*]Corresponding author: Jl. Salemba Raya No. 6, Central Jakarta 10430, Indonesia. Email: a_lusinanto@yahoo.com

ABSTRACT

Background: Although convalescent plasma contains neutralizing anti-SARS-CoV-2 antibodies, co-existing inflammatory mediators pose safety risks in critically ill recipients. Purified IgG preparations offer a safer alternative by concentrating therapeutic antibodies while eliminating these harmful components.

Objective: To establish a systematic protocol for purifying total IgG from SARS-CoV-2 convalescent serum using sequential chromatographic techniques.

Methods: Serum from 90 RT-PCR-confirmed recovered donors was pooled into three independent samples. Purification employed four sequential steps: ammonium sulfate precipitation (50% saturation), Sephadex G-100 size-exclusion chromatography, DEAE-Cellulose ion-exchange chromatography, and Protein A affinity chromatography. Purity and identity of IgG fractions were assessed by native polyacrylamide gel electrophoresis and radial immunodiffusion.

Results: Starting from serum containing 19.68 ± 7.27 mg/mL IgG and 110.47 ± 11.99 mg/mL total protein, the four-step purification yielded a final IgG concentration of 1.14 ± 0.70 mg/mL with total protein of 1.19 ± 0.16 mg/mL, representing 6.3-fold purification with a final IgG-to-total protein purity ratio of 1.01 ± 0.38 and an overall recovery of 5.8%. Native PAGE confirmed high purity with a single dominant IgG band.

Conclusion: Sequential chromatography yielded near-homogeneous IgG from SARS-CoV-2 convalescent serum, offering a laboratory-scale approach for preparing safer immunoglobulin therapeutics.

Keywords: convalescent serum, IgG purification, affinity chromatography, SARS-CoV-2

Introduction

Convalescent plasma therapy involves administering antibody-rich plasma from recovered patients to individuals suffering from the same infectious disease. This approach has been used successfully for over a century, with documented efficacy in treating tetanus, diphtheria, and hepatitis prior to the development of specific antiviral drugs [1,2]. During the SARS-CoV-2 pandemic, convalescent plasma emerged as a therapeutic option when specific antivirals and vaccines were not yet available [3].

However, the administration of whole convalescent plasma carries inherent risks. Beyond containing neutralizing antibodies against the pathogen, plasma includes numerous other proteins that may be harmful to recipients, particularly pro-inflammatory cytokines such as IL-1, IL-2, IL-6, IL-10, TNF-α, and IFN-γ. These cytokines can exacerbate inflammatory responses and contribute to severe complications in critically ill patients [4]. Clinical studies have documented adverse effects of convalescent plasma transfusion including transfusion-associated circulatory overload (TACO), transfusion-related acute lung injury (TRALI), and severe allergic reactions. In a large safety study of 5,000 hospitalized COVID-19 patients, the incidence of serious adverse events within four hours of transfusion was less than 1%, including TACO (0.18%), TRALI (0.1%), and severe allergic reactions (0.1%) [5]. COVID-19 patients with comorbidities who might be eligible for convalescent plasma treatment are at increased

risk of these adverse events, particularly TACO and TRALI [6]. Additionally, passive reporting systems may underestimate the true incidence of transfusion-associated complications, with active surveillance studies reporting TACO rates as high as 4.8-12% in certain patient populations [7].

Moreover, systematic reviews and meta-analyses of COVID-19 convalescent plasma therapy have reported inconsistent efficacy outcomes. Multiple randomized controlled trials have demonstrated that convalescent plasma treatment was not associated with reduced 28-day mortality (risk ratio 0.98, 95% CI 0.91-1.06) or improved clinical outcomes compared to standard care [8]. A comprehensive systematic review analyzing over 10,000 patients found no significant association between convalescent plasma treatment and decreased all-cause mortality (risk ratio 0.93, 95% CI 0.63-1.38) with low certainty of evidence [9]. These inconsistent results have been partly attributed to the variable composition of whole plasma, differences in neutralizing antibody titers among donors, and the presence of inflammatory mediators that may counteract the therapeutic benefits of neutralizing antibodies [10,11].

Purifying specific antibody fractions from convalescent plasma therefore offers a safer therapeutic alternative by concentrating beneficial immunoglobulins while eliminating immune-activating plasma proteins that may counteract therapeutic benefit [12-14]. Several studies have demonstrated that purified immunoglobulin preparations from convalescent donors provide equivalent or superior viral neutralization capacity compared to whole plasma while exhibiting significantly reduced inflammatory potential. A recent study produced highly purified anti-SARS-CoV-2 intravenous immunoglobulin containing 97.6% IgG with a 9.4-fold concentration of neutralizing antibodies compared to the source plasma [15]. Preclinical studies have confirmed that purified convalescent human IgG confers dose-dependent protection against SARS-CoV-2 replication and disease in animal models, with purity levels reaching 98.3% [16].

Compared to whole convalescent plasma transfusion, purified hyperimmune immunoglobulin preparations offer several distinct advantages: lower infusion volumes, easier administration routes, simpler storage requirements, higher and standardized neutralizing antibody titers, enhanced pathogen safety through dedicated virus inactivation and removal steps during manufacturing, reduced allergic and anaphylactic reactions, elimination of ABO and Rh compatibility requirements, and the absence of complement proteins and coagulation factors [17,18]. The purification process enables removal of IgA, IgM, albumin, fibrinogen, prekallikrein, and high-molecular-weight IgG aggregates that are associated with adverse reactions [19]. Clinical trial data have demonstrated that purified anti-SARS-CoV-2 intravenous immunoglobulins are safe, increase survival rates, and reduce disease progression risk in COVID-19 patients [20]. The depletion of immunologically active contaminants during purification preserves antibody functionality while minimizing the risk of immune-mediated complications that can occur with whole plasma administration [21,22].

Immunoglobulin G constitutes the predominant antibody class in serum and represents the primary humoral immune response following viral infection or vaccination. In SARS-CoV-2 infection, specific IgG antibodies develop during the recovery phase and can persist for months, making convalescent serum an attractive source for therapeutic antibody preparation [23,24]. Total IgG preparations, while containing a heterogeneous mixture of antibodies with various specificities, provide the advantage of concentrating all IgG including virus-specific antibodies while maintaining the natural antibody repertoire that may offer broader immunomodulatory benefits beyond viral neutralization.

Several approaches exist for purifying immunoglobulins from serum. Industrial-scale methods such as the Cohn cold ethanol fractionation process have been used for decades to produce intravenous immunoglobulin preparations [25]. More

recent protocols employ caprylic acid precipitation or direct affinity chromatography to streamline the purification process [26]. However, many of these methods require specialized equipment or are optimized for large-scale production rather than laboratory-scale preparation. Sequential chromatographic approaches combining salting-out, size-exclusion, ion-exchange, and affinity chromatography offer a systematic strategy for IgG purification that can be implemented with standard laboratory equipment [27].

Protein A affinity chromatography represents the most specific method for IgG purification, exploiting the natural interaction between Protein A from *Staphylococcus aureus* and the Fc region of IgG molecules [28]. When immobilized on a solid support such as agarose, Protein A selectively binds IgG while allowing other serum proteins to pass through unretained. Bound IgG can subsequently be eluted under mild acidic conditions that disrupt the Protein A-Fc interaction without permanently denaturing the antibody. This high specificity makes Protein A affinity chromatography the gold standard final purification step for therapeutic-grade IgG preparations.

Despite the clinical interest in convalescent plasma therapy during the COVID-19 pandemic, few studies have systematically documented laboratory-scale methods for purifying total IgG from SARS-CoV-2 convalescent serum. Most published protocols focus on either large-scale IVIG production or on purifying specific antibody subtypes such as secretory IgA from alternative sources like breast milk [29]. A detailed, reproducible protocol using conventional chromatographic techniques would therefore be valuable for research laboratories and smaller institutions seeking to prepare therapeutic immunoglobulin fractions without industrial-scale infrastructure, as well as for future emerging infectious disease scenarios where convalescent antibody therapy may be required.

The objective of this study was to establish and validate a systematic protocol for purifying total IgG from SARS-CoV-2 convalescent serum using sequential chromatographic separation techniques. We employed a four-step purification strategy consisting of ammonium sulfate precipitation, Sephadex G-100 size-exclusion chromatography, DEAE-Cellulose ion-exchange chromatography, and Protein A affinity chromatography. Purity and identity of IgG fractions at each purification step were monitored using native polyacrylamide gel electrophoresis and radial immunodiffusion.

Methods

Ethics statement and sample collection

This research was approved by the Ethics Committee under protocol number ND-798/UN.F1/ETIK/PPM.00.02/2023 in accordance with institutional guidelines and the Declaration of Helsinki.

Serum samples were obtained from 90 RT-PCR-confirmed virus-free individuals who had recovered from SARS-CoV-2 infection at least 14 days prior to blood collection. Three independent pools (P001, P002, P003) were prepared by combining equal volumes (3 mL) from 30 different donors, yielding 90 mL per pool. Samples were stored at -80°C until use.

IgG purification

The four-step purification protocol is explained below.

Step 1: Ammonium sulfate precipitation. Globulin fractions were precipitated by adding solid ammonium sulfate to pooled serum to achieve 50% saturation (313 g/L). After stirring for 2 hours at 4°C, the mixture was centrifuged at 10,000 × g for 30 minutes. The precipitate was resuspended in physiological saline and dialyzed against 100-fold excess saline using 12-14 kDa molecular weight cutoff tubing with buffer changes every 8 hours for 24 hours. Complete desalting was confirmed by negative $BaCl_2$ test. The dialysate volume was reconstituted to the original serum volume with saline.

Step 2: Sephadex G-100 size-exclusion chromatography. The desalted globulin fraction was applied to a Sephadex G-100 column (2.5 × 60 cm,

approximately 295 mL bed volume) pre-equilibrated with 0.01 M phosphate buffer containing 0.15 M NaCl, pH 7.2. The column was eluted at 30 mL/hour, collecting 2 mL fractions. Protein content was monitored at 280 nm using the Warburg-Christian method [30-32]. The first peak, corresponding to high molecular weight proteins including IgG (approximately 150 kDa), was pooled, concentrated by ultrafiltration using a 30 kDa cutoff membrane, and reconstituted to the original volume with 0.01 M phosphate buffer, pH 7.2.

Step 3: The IgG-enriched fraction was loaded onto a DEAE-Cellulose column (1.5 × 20 cm, approximately 35 mL bed volume) pre-equilibrated with 0.01 M phosphate buffer, pH 7.2. At this pH, IgG molecules exhibit a net positive charge, as the isoelectric points of the major IgG subclasses (IgG1–IgG4) range from approximately 6.5 to 9.5, placing the majority of IgG species above the operational pH and thus unretained by the positively charged DEAE matrix. While charge heterogeneity exists among IgG subclasses and glycoforms, the bulk of total IgG passed through unretained, whereas negatively charged serum proteins were selectively adsorbed onto the resin. The column was eluted at 20 mL/hour, collecting 2 mL fractions monitored at 280 nm. The first unbound peak containing IgG was pooled for affinity purification.

Step 4: Protein A affinity chromatography. Final purification was performed using a 5 mL Protein A-agarose column (Thermo Scientific Protein A IgG Purification Kit, Lot No. YK385951) equilibrated with binding buffer (0.02 M sodium phosphate, pH 7.0). The sample was loaded at 1 mL/minute, followed by washing with binding buffer until A_{280} reached baseline (<0.05). Bound IgG was eluted with 0.1 M glycine-HCl, pH 2.8, collecting 1 mL fractions into tubes containing 100 µL of 1 M Tris-HCl, pH 9.0, for immediate neutralization. Elution continued until absorbance returned to baseline. Fractions with A_{280} > 0.5 were pooled and stored at 4°C.

Protein quantification and purity assessment

Total protein concentration was determined using the Warburg-Christian method with absorbance measurements at 280 nm and 260 nm, calibrated against bovine serum albumin standards (0.1-2.0 mg/mL) [30-32]. IgG concentration was measured by Mancini-Carbonara single radial immunodiffusion using commercial plates (Kent Laboratories Inc., Lot No. 1014A23) containing 1.5% agarose in 0.1 M phosphate buffer, pH 7.0, with anti-human IgG antiserum [12]. Sample wells were filled with 5 µL and incubated for 48 hours at room temperature. Precipitin ring diameters were measured with 0.1 mm precision, and IgG concentrations were calculated from standard curves using reference IgG (0.5-4.0 mg/mL).

Protein purity was assessed by native PAGE on 7.5% polyacrylamide gels in Tris-glycine buffer, pH 8.3. Each lane was loaded with 20 µg total protein alongside native molecular weight markers (20-200 kDa). Electrophoresis was performed at 100 V for 3 hours at 4°C. Gels were stained with Coomassie Brilliant Blue R-250 and destained in methanol:acetic acid:water (4:1:5). Purification parameters were calculated as purity ratio (IgG concentration/total protein concentration), fold purification (purity ratio relative to starting serum), yield percentage, and recovery percentage.

Statistical analysis

Experiments were performed in triplicate using three independent pooled samples. Data are presented as mean ± SD. Differences between purification steps were assessed using one-way ANOVA with Tukey's post-hoc test. Statistical significance was set at $p<0.05$ using GraphPad Prism version 9.0.

Results

Initial characterization of pooled convalescent serum

The three pooled convalescent serum samples (P001, P002, P003) were characterized for baseline protein and IgG content (Table 1). Total protein concentrations measured 110.47 ± 11.99 mg/mL across the three pools. Initial IgG concentrations determined by radial immunodiffusion (Figure 1)

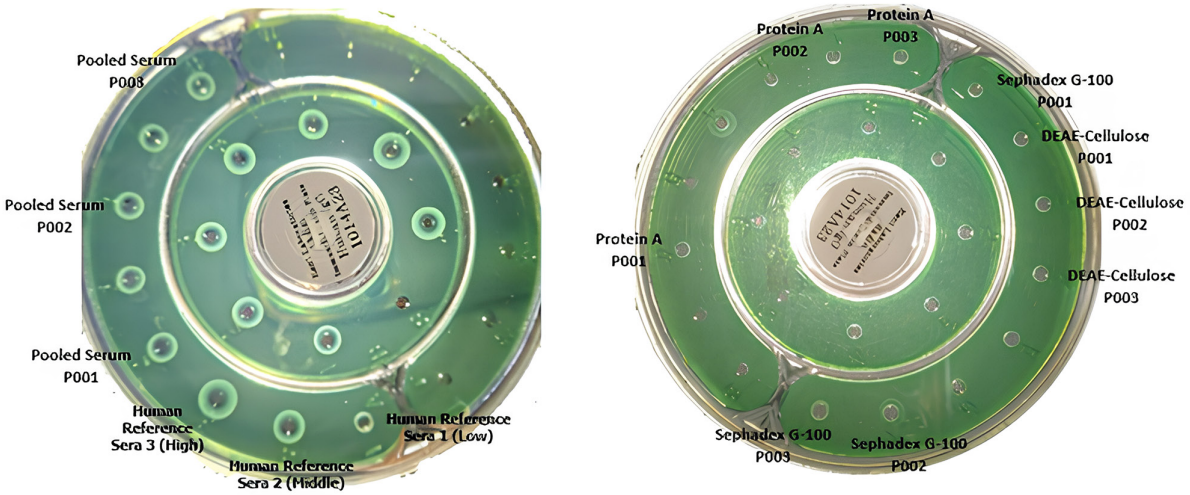

Figure 1. Radial immunodiffusion assay for IgG quantification. Wells were loaded with samples from each purification step. Agarose gels containing 0.1 M phosphate buffer (pH 7.0) and anti-human IgG antisera produced precipitation rings, whose diameters correspond to IgG concentration

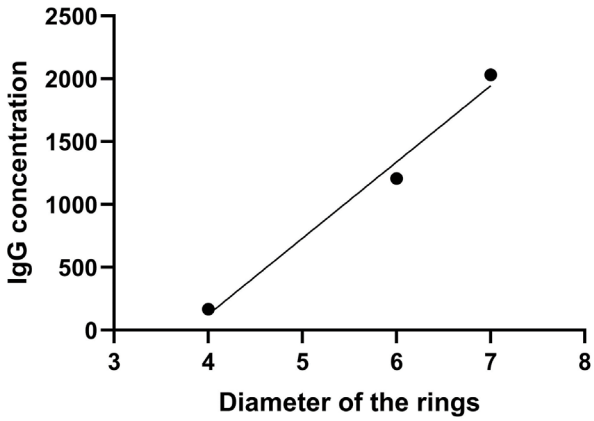

Figure 2. Calibration curve for IgG quantification by radial immunodiffusion. Ring diameters of reference sera were plotted against the logarithm of IgG concentration to generate a standard curve used for determining sample IgG levels.

were 19.68 ± 7.27 mg/mL, with individual values of 20.32 mg/mL (P001), 12.08 mg/mL (P002), and 26.64 mg/mL (P003). The baseline purity ratio (IgG to total protein) averaged 0.16 ± 0.05 (range: 0.11-0.20), indicating that IgG constituted approximately 11-20% of total serum proteins in the starting material.

IgG concentrations were determined by converting precipitin ring diameters to concentration values using a calibration curve generated from reference IgG standards (Figure 2). The calibration curve demonstrated excellent linearity ($r^2 > 0.98$) across the concentration range of 0.5-4.0 mg/mL.

Ammonium sulfate precipitation

Ammonium sulfate precipitation at 50% saturation effectively separated the globulin fraction from albumin and other soluble proteins. Following dialysis and volume reconstitution to the original serum volume (90 mL), protein analysis revealed changes in composition (Table 1). Total protein concentration decreased to 78.45 ± 22.16 mg/mL (29% reduction from starting serum), while IgG concentration was 14.42 ± 8.65 mg/mL. The purity ratio increased modestly to 0.19 ± 0.05, representing a 1.2-fold purification. This step achieved an average recovery of 73.2% of initial IgG, with absolute IgG masses of 1,297 mg, 885 mg, and 1,942 mg recovered from P001, P002, and P003, respectively.

Sephadex G-100 size-exclusion chromatography

Size-exclusion chromatography produced distinct elution profiles with two major peaks (Figure 3). The first peak, eluting at the void volume (fractions 8-18, corresponding to approximately 16-36 mL elution volume), contained high molecular weight proteins including IgG (approximately 150 kDa). The second peak (fractions 22-35) contained lower molecular weight proteins. Fractions within the first peak showing $A_{280} > 0.3$ were pooled and

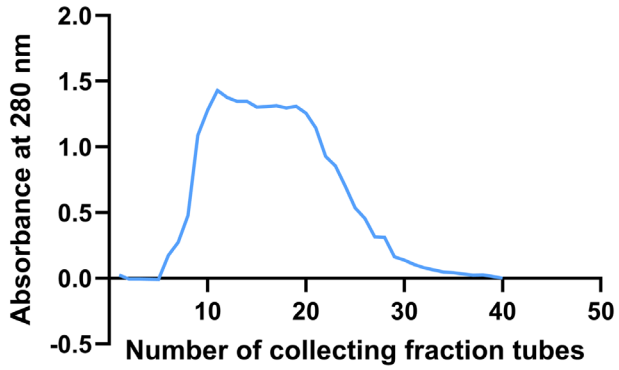

Figure 3. Elution profile from Sephadex G-100 size-exclusion chromatography. The first peak represents high molecular weight proteins including IgG (approximately 150 kDa). The second peak contains lower molecular weight proteins. Absorbance was measured at 280 nm for each 2 mL fraction.

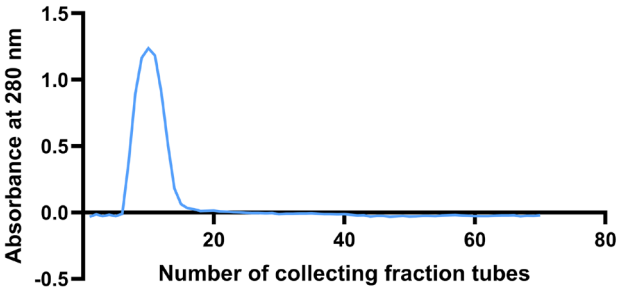

Figure 4. Elution profile from DEAE-Cellulose ion-exchange chromatography. The first peak contains positively charged proteins (including IgG) that did not bind to the positively charged resin at pH 7.2. The second peak represents negatively charged proteins retained and subsequently eluted. Absorbance measured at 280 nm.

concentrated by ultrafiltration, then reconstituted to the original volume (90 mL).

After pooling and concentration, this fraction showed total protein of 4.55 ± 0.71 mg/mL and IgG concentration of 2.01 ± 1.73 mg/mL (Table 1). The purity ratio was 0.45 ± 0.32, representing 2.8-fold purification relative to starting serum. Considerable variation was observed among the three pools: P002 showed a purity ratio of 0.91, while P001 and P003 showed 0.12 and 0.31, respectively. This variation likely reflected differences in the initial serum composition among donor pools. Overall recovery at this step was 10.2% with absolute IgG masses of 42 mg (P001), 350 mg (P002), and 151 mg (P003).

DEAE-Cellulose ion-exchange chromatography

Ion-exchange chromatography separated proteins based on their charge properties at pH 7.2. The elution profile (Figure 4) showed two distinct peaks. The first peak represented unbound, positively charged proteins (including IgG) that did not bind to the DEAE-Cellulose matrix and eluted in the flow-through and early wash fractions (fractions 3-10). The second peak contained negatively charged proteins that were retained on the column.

The pooled first peak (fractions 3-10 with A_{280} > 0.4, approximately 16 mL total) was concentrated to 5 mL for subsequent affinity purification. Analysis of this fraction showed total protein concentration of 1.32 ± 0.16 mg/mL with IgG at 0.47 ± 0.00 mg/mL (Table 1), achieving a purity ratio of 0.36 ± 0.06. This represented 2.3-fold purification relative to starting serum. The cumulative recovery was 2.4% with absolute IgG masses of 42 mg (P001), 42 mg (P002), and 42 mg (P003). Note that the apparently lower purity ratio compared to the Sephadex G-100 step does not indicate loss of purity, but rather reflects the concentration-based calculation method and the fact that contaminating proteins were selectively removed through retention on the column rather than being co-purified with IgG.

Protein A affinity chromatography

Affinity chromatography using Protein A-agarose provided the highest degree of purification and specificity. The chromatogram (Figure 5) showed two distinct peaks. The first peak represented unbound proteins that flowed through during sample loading and washing. The second, sharp peak was obtained after elution with pH 2.8 glycine-HCl buffer and contained specifically bound IgG.

Fractions within the elution peak showing A_{280} > 0.5 (typically fractions 16-21, representing 5-7 mL) were pooled. This final purified fraction had total protein concentration of 1.19 ± 0.16 mg/mL and IgG concentration of 1.14 ± 0.70 mg/mL (Table 1), yielding a purity ratio of 1.01 ± 0.38. Individual purity ratios for P001, P002, and

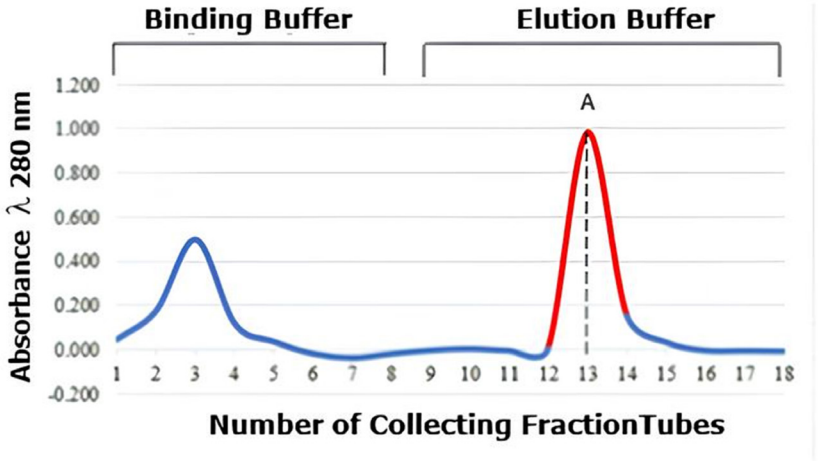

Figure 5. Affinity chromatography profile using Protein A-agarose. Peak 1 contains unbound proteins that flowed through during sample loading and washing. Peak 2, eluted with pH 2.8 glycine-HCl buffer, contains specifically bound IgG. The sharp peak indicates high binding specificity of Protein A for IgG.

Table 1. Purification summary showing total protein concentration, IgG concentration, purity ratio (IgG/total protein), and fold purification at each step for three independent pooled serum samples (P001, P002, P003).

Sample	Isolation and purification steps	Total protein (mg/mL)	IgG concentration (mg/mL)	Ratio (IgG:Protein)
P001	Pooled serum	110.47	20.32	0.18
	Sephadex G-100 (1th peak)	3.97	0.47	0.12
	DEAE-Cellulose (1th peak)	1.37	0.47	0.34
	Protein A	1.14	1.26	1.11
P002	Pooled serum	107.64	12.08	0.11
	Sephadex G-100 (1th peak)	4.29	3.89	0.91
	DEAE-Cellulose (1th peak)	1.45	0.47	0.33
	Protein A	1.37	0.47	0.34
P003	Pooled serum	131.59	26.64	0.20
	Sephadex G-100 (1th peak)	5.39	1.68	0.31
	DEAE-Cellulose (1th peak)	1.14	0.47	0.42
	Protein A	1.06	1.68	1.58

Data demonstrate progressive increase in purity ratio from 0.16-0.20 in starting serum to 1.01-1.58 in final affinity-purified product, with overall 6.3-fold purification and 5.8% recovery.

P003 were 1.11, 0.34, and 1.58, respectively. A purity ratio approaching 1.0 indicated that IgG comprised essentially all detectable protein in the final preparation. The overall purification achieved 6.3-fold enrichment compared to starting serum. Final recovery was 5.8% of initial IgG, with absolute final IgG masses of 113 mg (P001), 42 mg (P002), and 151 mg (P003).

Table 1 summarizes the protein and IgG concentrations, purity ratios, and fold purification for each step across all three pooled samples. The progressive increase in purity ratio from 0.16 ± 0.05 in starting serum to 1.01 ± 0.38 in the final product demonstrated effective separation of IgG from contaminating proteins. However, the modest final recovery (5.8%) reflected cumulative losses during multiple purification steps, particularly during concentration, buffer exchange, and column chromatography procedures where IgG may be lost through non-specific adsorption to membranes and matrices, incomplete elution, or handling losses during fraction collection and pooling.

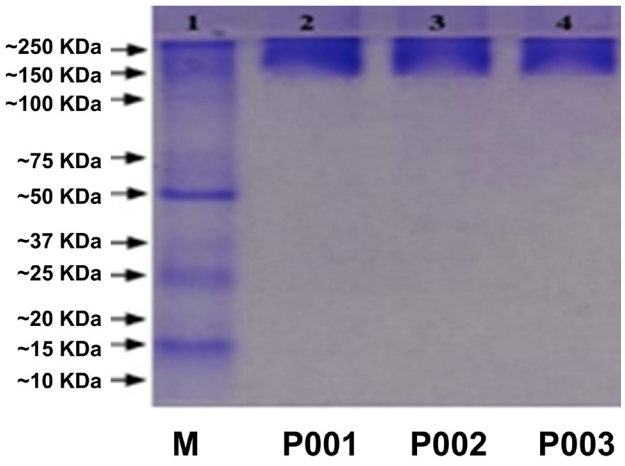

Figure 6. Native PAGE analysis of purification steps. Lane 1: Molecular weight markers (20-200 kDa). Lane 2: Starting pooled serum showing multiple protein bands. Lane 3: Post-ammonium sulfate precipitation. Lane 4: Post-Sephadex G-100 chromatography. Lane 5-7: Final affinity-purified IgG from pools P001, P002, and P003, respectively, showing single dominant bands at approximately 148 kDa near the origin due to the positive charge of IgG.

Native PAGE analysis confirms IgG purity

Native PAGE analysis confirmed the progressive purification of IgG (Figure 6). Starting serum showed multiple protein bands representing the complex mixture of serum proteins. After ammonium sulfate precipitation, the band pattern simplified but still contained several contaminating proteins. Size-exclusion and ion-exchange chromatography further reduced contaminating bands. The final affinity-purified product from all three pooled samples (lanes 5, 6, and 7) displayed a single dominant band that remained near the origin, consistent with the high molecular weight (approximately 148 kDa) and positive charge of IgG under native conditions. Comparison with molecular weight markers confirmed the purified protein corresponded to the expected size of IgG (between 100-150 kDa markers). The absence of additional bands in the final product indicated successful removal of non-IgG proteins and confirmed the high purity achieved through affinity chromatography.

The electrophoretic mobility pattern, combined with immunodiffusion results showing specific reactivity with anti-human IgG antibodies, verified that the purified protein was indeed IgG. However, this total IgG preparation contained a heterogeneous mixture of antibodies with various specificities, of which anti-SARS-CoV-2 antibodies represented only a fraction.

Discussion

This study purified total IgG from SARS-CoV-2 convalescent serum using a sequential four-step chromatographic approach, achieving 6.3-fold purification with a final purity ratio of 1.01. The strategy combined classical protein separation techniques with specific affinity chromatography to minimizing contamination from other serum proteins, particularly inflammation-associated mediators that could pose safety concerns in therapeutic applications.

The choice of 50% ammonium sulfate saturation for initial precipitation effectively separated globulins from albumin, which remains soluble at this concentration. This approach differs from the cold ethanol fractionation method developed by Cohn et al. (1946), which, although suitable for large-scale industrial processing, requires specialized equipment and precise temperature control [25]. Our method offers a more accessible alternative for smaller-scale laboratory purification while maintaining adequate separation efficiency. The 73.2% recovery at this step was comparable to standard globulin precipitation protocols, though the modest 1.2-fold purification confirmed that this serves primarily as a bulk separation rather than a refined purification step.

Size-exclusion chromatography provided molecular weight-based separation, effectively isolating high molecular weight proteins including IgG (approximately 150 kDa) from smaller serum proteins. However, this step resulted in substantial losses (10.2% cumulative recovery), primarily attributable to sample dilution during elution and incomplete recovery during the concentration process. The high variability observed among the three pooled samples at this stage reflects inherent differences in serum composition from different donor populations, emphasizing the importance of pooling strategies in standardizing therapeutic preparations.

Ion-exchange chromatography exploited the charge properties of IgG, which carries a net positive charge at physiological pH due to its isoelectric point range of 6.5-8.5. The DEAE-Cellulose resin retained negatively charged proteins while allowing IgG to flow through unretarded. Although this step appeared to decrease the purity ratio in some samples, this reflects the limitations of concentration-based calculations when proteins are selectively removed rather than retained with the product. The primary value of this step was removing acidic protein contaminants that would otherwise interfere with subsequent affinity purification.

Protein A affinity chromatography provided the highest degree of specificity, exploiting the unique interaction between Protein A from *Staphylococcus aureus* and the Fc region of IgG [28]. This interaction is highly specific and reversible, allowing selective capture of IgG while non-IgG proteins pass through unbound. The elution at pH 2.8 disrupts the Protein A-Fc interaction through conformational changes without permanently denaturing IgG, provided the pH is rapidly neutralized. This final step achieved near-complete purity (purity ratio 1.01), with native PAGE confirming a single dominant band and radial immunodiffusion verifying IgG identity.

The overall recovery of 5.8% was lower than reported in some commercial IVIG preparation protocols, which achieve 40-60% recovery [12, 13, 26]. This discrepancy stems from several factors. First, our multi-step approach included two concentration steps that inevitably result in losses through membrane adsorption and incomplete recovery. Second, the mild elution conditions used to preserve antibody activity may have left some IgG bound to the Protein A matrix. Third, we prioritized purity over yield, accepting lower recovery to ensure removal of potentially harmful contaminating proteins. Industrial processes often employ single-step caprylic acid precipitation or optimized affinity chromatography with higher recovery rates, though these may sacrifice some degree of purity [26].

The purified total IgG preparation contains a heterogeneous mixture of antibodies reflecting the complete immunological history of the donors, not exclusively anti-SARS-CoV-2 antibodies. Specific anti-SARS-CoV-2 antibodies represent only a minor fraction of the total IgG in convalescent serum, as the polyclonal IgG pool reflects the donor's complete immunological history encompassing responses to all previously encountered pathogens and self-antigens. For therapeutic applications requiring only SARS-CoV-2-specific antibodies, an additional immunoaffinity chromatography step using immobilized SARS-CoV-2 spike protein or receptor-binding domain would be necessary. However, such purification would further reduce yield and may not be necessary for all applications. Total IgG from convalescent donors may provide broader benefits through non-specific immunomodulatory effects beyond viral neutralization [21, 22].

Comparison with alternative approaches reveals both advantages and limitations of our methodology. Mane et al. (2024) recently reported direct affinity purification of secretory IgA from breast milk using 65% ammonium sulfate precipitation followed immediately by affinity chromatography [29]. While this streamlined approach offers higher recovery, breast milk contains far fewer contaminating proteins than serum, making intermediate purification steps less critical. Our serum-based approach required additional chromatographic steps to achieve comparable purity due to the complexity of the starting material.

The quantification method used here, radial immunodiffusion, offers adequate accuracy for process monitoring and requires only basic laboratory equipment. However, it is less precise than immunonephelometric or ELISA-based methods [33]. The 48-hour incubation time, while lengthy, allowed the purification process to continue uninterrupted. For large-scale production or quality control requiring rapid turnaround, alternative quantification methods would be preferable despite higher equipment costs.

Several limitations warrant acknowledgment. First, we did not assess the functional activity

of purified IgG through neutralization assays or specific anti-SARS-CoV-2 ELISA, making it impossible to determine whether the purification process preserved antibody function or what proportion of the final product represented virus-specific antibodies. Second, we did not perform Western blot analysis or SDS-PAGE under reducing conditions, which would have confirmed the integrity of IgG heavy and light chains and detected any proteolytic degradation. Third, the stability of purified IgG during storage was not evaluated, leaving questions about optimal storage conditions and shelf life unanswered. Finally, our small-scale laboratory process may not translate directly to clinical-scale production without significant optimization.

Despite these limitations, this study demonstrates that high-purity total IgG can be successfully isolated from SARS-CoV-2 convalescent serum using conventional chromatographic techniques accessible to most research laboratories. The approach provides a safer alternative to whole convalescent plasma by removing potentially harmful pro-inflammatory cytokines while concentrating therapeutic antibodies. The methodology could be adapted for purifying IgG from convalescent serum of other infectious diseases where specific antiviral therapeutics are unavailable or during early stages of emerging infectious disease outbreaks.

Conclusion

We successfully purified total IgG from SARS-CoV-2 convalescent serum through sequential ammonium sulfate precipitation, size-exclusion chromatography, ion-exchange chromatography, and Protein A affinity chromatography. The final product achieved 6.3-fold purification with a purity ratio of 1.01, indicating that IgG comprised essentially all protein in the preparation. Native PAGE analysis confirmed high purity with a single dominant band corresponding to IgG. Although the overall recovery of 5.8% was modest, the methodology successfully removed contaminating proteins including potentially harmful inflammatory mediators, by reducing the burden of co-purified immunostimulatory proteins. This approach provides an accessible purification strategy for laboratories seeking to prepare immunoglobulin therapeutics from convalescent serum, though further functional characterization and optimization for clinical-scale production would be necessary for therapeutic applications. Future work should include specific anti-SARS-CoV-2 antibody quantification, neutralization assays, and immunoaffinity purification to isolate virus-specific antibodies for targeted therapeutic use.

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Declaration of interest

The authors declare that none of them has any conflict of interest with any private, public, or academic party related to the information contained in this manuscript.

Author contributions

AL, RSEG, DRG, MS conceptualization, methodology, investigation. AL, RSEG data curation. DRG, MS formal analysis. AL, RSEG, DRG writing original draft. MS writing review and editing. MS supervision.

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