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Intravenous Immunoglobulins: Evolution of Commercial IVIG Preparations

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KEYWORDS

• Intravenous immunoglobulin • Primary immunodeficiency
• Manufacture • Commercial

Human immunoglobulin G (IgG) has been used to treat people with inherited immunoglobulin deficiencies since 1952 when Bruton[1] infused a child with undetectable ''gamma globulin'' levels and who suffered from recurrent pneumococcal infections. Subcutaneous infusions of 3.2 g/mo produced measurable gamma globulin levels and completely eliminated pneumococcal infections. Human IgG soon became the standard treatment for patients with primary antibody deficiencies who develop chronic bacterial infections.

The first human IgG produced on a large scale was known as immune serum globulin or ISG. It was produced by a cold ethanol precipitation process developed in the early 1940s by E. J. Cohn and his coworkers[2,3] in the Department of Physical Chemistry at Harvard Medical School. ISG was formulated at a protein concentration of 165 mg/mL that contained 0.3 Molar glycine, 0.9% (weight/volume) sodium chloride and 0.1 g/L merthiolate. ISG solutions were adjusted to pH 6.8 $\pm$ 0.4 and stored at 5°C. With time, ISG solutions tended to form particles (aggregates) during storage. Aggregates were generally believed to be the cause of adverse events when ISG was injected intravenously. Therefore, the first commercial immunoglobulins were restricted to intramuscular or subcutaneous injections.

INTRAVENOUS IMMUNOGLOBULIN

In 1962, spontaneous complement activation (anticomplement activity) by IgG aggregates was proposed as the principal cause of adverse side reactions when ISG was injected intravenously.[4] The desire to eliminate anticomplement activity

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Immunol Allergy Clin N Am 28 (2008) 765–778
doi:10.1016/j.iac.2008.06.002 **immunology.theclinics.com**
0889-8561/08/$ – see front matter

had a significant impact on intravenous immunoglobulin (IVIG) development.[5,6] Some manufacturers reduced anticomplement activity by enzymatic digestion or chemical modification. The first IVIG was produced by pepsin digestion and contained a principal fragment with two antigen binding sites linked by disulfide bonds.[7]

The desire to reduce anticomplement activity and produce IVIGs with ''intact'' IgG as the principal component led some manufacturers to limit pepsin treatments,[4] to use the more specific enzyme plasmin,[8] and to chemically modify the product. Chemically modified IVIGs were produced that were structurally intact, were low in anticomplement activity, and contained no IgG fragments.[9–11]

Treatments of immunoglobulins with enzymes and chemical modification to suppress spontaneous complement activation had several unintended consequences. The treatments also reduced important antibody biological activities required for clinical efficacy. For example complement activation by antigen-antibody complexes plays an important role in the killing of encapsulated bacteria by leukocytes.[12] Antibodies that are chemically and physically altered are rapidly removed from the circulatory system by the reticuloendothelial system. Thus some antibodies in enzyme-digested and chemically modified IVIGs were shown to have reduced bacterial opsonizing activities[12–15] and shortened circulating half-lives.[16–18]

SECOND GENERATION INTRAVENOUS IMMUNOGLOBULIN

All commercial IVIGs are produced from large pools of human plasma by first concentrating the IgG by cold ethanol fractionation. Although IgG produced by cold ethanol fractionation is relatively pure, it contains trace amounts of highly active contaminants that have the potential to cause most of the adverse events previously attributed to aggregates. These contaminants include prekallikrein activator (which initiates production of the potent vasodilator bradykinin), prekallikrein, activated coagulation factors, complement proteins, and immunoglobulins A and M.[6] Other contaminants such as plasmin and plasminogen can degrade IgG to form split products and to reduce some antibody activities during ISG storage.[19]

The desire to produce IVIG that contain native IgG with antibodies that are fully active led to development of IVIG using purification with anion exchange (DEAE) chromatography. The first purified IVIG contained none of the trace contaminants associated with adverse events. Some antibody biological activities such as bacterial opsonization and virus neutralization were higher than in treated products.[6] Now virtually all commercial IVIGs are produced with an anion exchange chromatography step and contain relatively low levels of trace contaminants.

Historically, IVIGs were freeze-dried to obtain a preparation that would be stable for 2 to 3 years. In 1986, McCue and coworkers[20] reported that adjusting the pH to 4.25 produced a clear, physically stable IgG solution. Clinical studies demonstrated that patients tolerated IgG solutions formulated at a pH significantly lower than the customary range of 6.4 to 7.2.[21] This product represented a major advance in IVIG product formulation.

Table 1 lists commercial IVIG preparations currently (or soon to be) available in North America. Of the nine products licensed in the United States, seven are produced by cold ethanol fractionation followed by purification using ion exchange chromatography. Seven products are formulated as liquids and two are freeze-dried. All are produced with specific virus inactivation or removal steps incorporated into their manufacturing procedures.

DEVELOPMENT OF VIRUS ELIMINATION PROCEDURES

Transmission of ''homologous serum hepatitis'' through whole blood, plasma, and serum was a great concern during development of human plasma proteins.[1] Yellow fever vaccines stabilized with human serum had produced 23,000 cases of hepatitis in military personnel. Pooled human plasma presented a higher risk of hepatitis transmission than whole blood because of the increased probability that pooled plasma would be contaminated by one or more donors. Human albumin solutions were also responsible for hepatitis transmission.[22]

Heat can be used to inactivate viruses and proteins. The destruction temperature of a protein is sharply defined and is different for each protein.[23] In the presence of substrate, enzymes can be heated to temperatures 10 degrees higher than in the absence of substrate.[23] In 1948, Gellis and coworkers[22] reported that hepatitis transmission by albumin was eliminated by heating it for 10 hours at 60°C. Virus inactivation of albumin by heat treatment was possible because of the discovery that addition of stabilizers increased the heat resistance of albumin. Human albumin has many binding sites for hydrophobic molecules and plays a major role in the transport of fatty acids. Filling these sites with the stabilizers acetyltryptophan and caprylic acid allows albumin to withstand heating for 10 hours at 60°C. Since albumin has no measurable biological activity, the full impact of heating albumin is not known.

Unfortunately, other plasma proteins in solution are inactivated by heat and early attempts to inactivate viruses in high risk plasma products were unsuccessful. High risk plasma products included fibrinogen, Factor VIII concentrate, and Factor IX.[24] Heated albumin solutions and immunoglobulins produced by cold ethanol fractionation were considered to be low-risk products.[24]

Factor VIII is rapidly inactivated when heated in solution. However, dried Factor VIII is relatively heat stable under certain conditions. This observation led to development of heat-treated Factor VIII preparations in the 1980s.[25,26] Fortunately, HIV was also inactivated in heated Factor VIII but the products had lower biological activities, were relatively insoluble, and produced a higher incidence of Factor VIII inhibitors. Unfortunately, non-A, non-B hepatitis was not inactivated.[25]

The perception that immunoglobulins produced by cold ethanol fractionation had a low risk of transmitting virus infections changed in 1983 when Lane reported that an experimental IVIG produced by cold ethanol fractionation transmitted non-A, non-B hepatitis.[27] During this same period, HIV was isolated and shown to be transmitted by blood and blood products.[28,29] The emergence of HIV and reports of non-A, non-B hepatitis transmission by some IVIG products[30,31] caused manufacturers and regulatory agencies to examine existing IVIG manufacturing procedures for their capacity to eliminate viruses.[32–41] Development of dedicated virus inactivation procedures for IVIG production was also initiated.[42,43]

VIRUS INACTIVATION OF INTRAVENOUS IMMUNOGLOBULIN

Studies of IVIG manufacturing procedures revealed that cold ethanol fractionation removes viruses by two mechanisms: inactivation and partitioning. Several laboratories demonstrated that HIV is inactivated by cold ethanol under conditions used in IVIG production.[33–36,41] However, vesicular stomatitis virus and Sindbis virus, both used as models for the hepatitis C virus (HCV), formerly known as non-A, non-B, were stable under similar conditions.[41]

Given the success of heat treatment in producing albumin with a low-risk of transmitting hepatitis, heat treatments for IVIG were evaluated. One IVIG was stabilized with 33% (weight/weight) sorbitol at pH 5.5 and heated at 60°C for 10 hours.[43] Several

Table 1
Production and properties of commercial immunoglobulins

Trade Names	Manufacturer	Registrations	Manufacturing Procedure	Composition	Comments
Gammagard S/D	Baxter HealthCare Corp	United States, Canada, European Union	Cold ethanol fractionation, DEAE chromatography, S/D, pH 6.8 ± 0.4, freeze-dried	50 mg/mL; 8.5 mg/mL NaCl, 0.3 M glycine, 20 mg/mL PEG, 3 mg/mL albumin, 20 mg/mL glucose	<1 μg/mL IgA
Gammagard Liquid, KIOVIG	Baxter HealthCare Corp	United States, European Union	Cold ethanol fractionation, DEAE chromatography, S/D, nanofiltration, pH 4.85 ± 0.25, liquid	100 mg/mL; 0.25 M glycine	—
Intratect	Biotest	Germany, European Union	Cold ethanol fractionation, octanoic acid/calcium acetate treatment, S/D, liquid	50 mg/mL; 0.3 M glycine	—
Vigam	Bio Products Laboratory	England	—	50 mg/mL; IgG, 20 mg/mL human albumin, sucrose, glycine, pH 4.8–5.1	In US clinical trials (Gammaplex)
Carimune NF	CSL Behring AG	United States, European Union	Cold ethanol fractionation, pepsin treatment, nanofiltration, pH 6.6 ± 0.2, freeze-dried	30. 60, 90 or 120 mg/mL; 100 mg/mL sucrose, 1.2 mg/mL NaCl	—
Sandoglobulin NF liquid Carimune NF liquid	CSL Behring AG	Canada	Cold ethanol fractionation, pepsin treatment, DEAE Sephadex batch adsorption, nanofiltration, pH 5.3 liquid	120 mg/mL; 100 mM L-isoleucine, 120 mM L-proline, 80 mM Nicotimamide	—

Privigen	CSL Behring AG	United States	Cold ethanol fractionation, octanoic acid fractionation, anion exchange chromatography, nanofiltration, pH 4.8 ± 0.2, liquid	100 mg/mL; 0.25 M proline	—
Vivaglobin	CSL Behring AG	United States	Cold ethanol, fatty alcohol, DEAE chromatography, activated carbon, heated 10 h @ 60°, pH 6.8 ± 0.4, liquid	160 mg/mL; 3 g/L NaCl, 0.25 N glycine	Formulated for subcutaneous injection
Flebogamma 5%	Instituto Grifols, SA	United States, Spain	Cold ethanol, polyethylene glycol precipitation, ion exchange chromatography, 10 h @ 60°, pH 5.5 ± 0.5, liquid	50 mg/mL; 50 mg/mL D-sorbitol, <6 mg/mL polyethylene glycol	—
Flebogamma 5% DIF	Instituto Grifols, SA	United States	Cold ethanol, polyethylene glycol precipitation, ion exchange chromatography, pH 4 @ 37°, 10 h @ 60°, S/D, nanofiltration, pH 5.5 ± 0.5, liquid	50 mg/mL; 50 mg/mL D-sorbitol, <3 mg/mL polyethylene glycol	4 virus elimination steps

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Table 1
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Trade Names	Manufacturer	Registrations	Manufacturing Procedure	Composition	Comments
Octagam	Octapharma Pharmazeutika Produktionsges.m.b.H.	United States, European Union	Cold ethanol fractionation, S/D, 24 h @ pH 4, pH 5.5 $\pm$ 0.4, liquid	50 mg/mL; 100 mg/mL maltose	—
Omr-IgG-am	Omrix Biopharmaceuticals Ltd	Israel	Cold ethanol fractionation, S/D, 24 h @ pH 4, pH 5.5 $\pm$ 0.4, liquid	50 mg/mL; 100 mg/mL maltose	In US clinical trials
Gamunex	Talecris Biotherapeutics, Inc	United States European Union	Cold ethanol fractionation, caprylate precipitation, Q Sepharose-ANX Sepharose chromatography, pH 4.25 $\pm$ 0.25, liquid	100 mg/mL; 0.2 M glycine	—

Abbreviations: IgA, immunoglobulin A; NF, nanofiltration; PEG, polyethylene glycol; S/D, solvent-detergent.

enveloped viruses and one nonenveloped virus were studied. All viruses were completely inactivated except for HCV. No substantial changes in IgG physicochemical and biological properties were reported.

In 1988, Horowitz[42] reported that a solvent-detergent process, originally developed to inactivate viruses in Factor VIII concentrates, was an effective virus inactivation process for IgG solutions. Solvent-detergent virus inactivation was rapidly adopted by several IVIG manufacturers (**Table 2**).

Inactivation of hepatitis C and bovine viral diarrhea virus (BVDV, a surrogate for HCV) was reported in liquid IVIG formulated at pH 4.25 and incubated for 21 days at 21°.[38] Pepsin digestion at pH 4 and 37° has also been shown to inactivate several enveloped viruses.[39,40] Recently, incubation of immunoglobulin solutions with caprylic acid has been shown to be an effective procedure for inactivating enveloped viruses.[44]

VIRUS REMOVAL (NANOFILTRATION)

Manufacturers have long known that clarification filtration of cold ethanol fractionation intermediates in the presence of a filter aid is an effective virus removal procedure. Some manufacturers have validated such processes as virus removal steps (see **Table 2**).

In 1994, Burnouf-Radosevich and colleagues[45] reported virus removal from Factor IX and Factor XI solutions by newly developed hollow fiber nanofiltration filters. The filters were composed of cellulose layers treated to produce mean pore sizes of 15 ± 2 and 35 ± 2 nanometers (nm). Virus spiking experiments demonstrated that a single dead-end filtration with the 35 nm filter removed >5.7 to 7.8 $\log_{10}$ of HIV-1, BVDV, porcine pseudorabies virus (PRV) reovirus type 3, simian virus 40 (SV 40), and bovine parvovirus, a small (18–25 nm) nonenveloped virus.[45]

Table 2
Dedicated virus inactivation and removal procedures used in IVIG production

Virus Inactivation/Removal Procedure	Product
Solvent-detergent inactivation	Gammagard S/D
	Gammagard Liquid
	Flebogamma 5% DIF
	Octagam
	Omr-IgG-am
Heat inactivation (10 h at 60°C)	Vivaglobin
	Flebogamma 5%
	Flebogamma 5% DIF
Removal by nanofiltration	Gammagard Liquid
	Carimune NF
	Privigen
pH 4 incubation (in process)	Flebogamma 5% DIF
	Octagam
	Omr-IgG-am
	Privigen
Low pH incubation in final container (21 d)	Gamunex
Low pH incubation at elevated temp in final container	Gammagard Liquid
Pepsin treatment	Carimune NF
Caprylic acid virus inactivation	Gamunex

In studies of immunoglobulin solutions with protein concentrations up to 12 mg/mL, O'Grady and colleagues[46] demonstrated that the 35 nm filter removed 6–7 $\log_{10}$ mouse type C retrovirus, SV 40 and PRV, whereas poliovirus was removed by only a 15 nm filter. Similar results were obtained with 70 mg/mL IgG solutions.[47]

Omar and Kempf[48] studied the effectiveness of nanofiltration to remove small non-enveloped viruses. The viruses studied were bovine enterovirus (BEV, ~30 nm), bovine parvovirus (BPV, ~18–25 nm) and minute virus of mice (MVM, ~18–25 nm). Nanofiltration was performed with filters having nominal pore sizes of 20 and 50 nm. Despite their small size, each virus was efficiently removed from 10 mg/mL IgG solutions. The authors demonstrated that removal of viruses smaller in diameter than the pore sizes of the nanofilter was due to antibodies bound to the viruses.[48] Nanofiltration has been adopted by several IVIG manufacturers (see **Table 2**).

DONOR SCREENING AND PLASMA TESTING

Concomitant with development of virus inactivation and removal procedures, scientists also recognized the importance of eliminating infected donors and developed more sensitive tests for blood-borne pathogens. Although people with illnesses are always excluded from donating blood or plasma, some donors do not feel sick or have clinical symptoms in the early stages of an infection. During this time (window period), blood or plasma donations may transmit an infection. Thus development of donor screening tests involved not only tests for new pathogens but also tests of ever increasing sensitivity to eliminate window period infections.

Gürtler[49] has reviewed blood-borne pathogens with respect to their relevance to transfusion. Relevant pathogens are considered to be human pathogens that cause chronic, progressive wasting, or lethal diseases; and some infectious agents that are not prevalent in the transfused population. By these criteria, hepatitis B virus (HBV), HCV, and human immunodeficiency viruses types 1 and 2 (HIV-1 and HIV-2) were characterized as relevant. Parvovirus B19, cytomegalovirus, and hepatitis A viruses (HAV) were classified as occasionally relevant. Since this review was published, nonenveloped viruses such as Parvovirus B19 and HAV have become more relevant and the Severe Acute Respiratory Syndrome-Corona virus (SARS-CoV) and West Nile virus (WNV) have emerged. Thus relevance of pathogens to transfusion is an evolving concept.

Given the early concern about hepatitis transmission, identification of hepatitis viruses and development of sensitive donor screening tests became a high priority. A sensitive test for HBV was developed in 1972[50] and was used to eliminate infected donors. Unfortunately, the HBV test did not eliminate transfusion-related hepatitis and the search for one or more non-A, non-B hepatitis viruses was initiated. The AIDS epidemic led to rapid development of a screening test for antibodies to human immunodeficiency virus (HIV-1) in 1984.[51] In 1989 the genome of a non-A, non-B hepatitis virus was isolated and used to develop a donor screening test for HCV.[52] Today, plasma is screened for antibodies to syphilis, HIV-1, HIV-2, and HCV, and for HBV and HIV antigens. Extremely sensitive tests for HCV, HIV-1, HBV, and parvovirus B19 nucleic acids have recently been introduced and are now being used to further eliminate window period donations.

PRION REMOVAL

The risk of transmitting prion diseases such as Creutztfedt-Jakob disease (CJD) or Variant Creutzfeldt-Jakob disease (vCJD) by transfusions of human blood or blood products is theoretical at this time. However, the incubation time for development of CJD disease is so long that it is difficult to quantify the risk.

There is enough uncertainty about the relationship of vCJD to bovine spongiform encephalopathy (BSE) that regulatory agencies have take steps to reduce the risk. Donors that have spent $\geq$6 months in the United Kingdom from 1986 to the present are not permitted to donate blood or plasma in the United States and Europe. The recent observation that BSE and scrapie are transmitted from sheep to sheep by transfusions may support this donor deferral program.

Trejo and colleagues[53] studied removal of hamster scrapie protein (PrP^{sc}) during two steps in IVIG production. Western Blot and infectivity tests demonstrated that PrP^{sc} was removed during two filtration steps. One of the steps was a depth filtration step that is common to all IVIG manufacturing procedures.

A similar study was performed by Gregori and colleagues.[54] Proteinase K resistant PrP (PrP^{res}) was determined by Western Blot analysis whereas infectivity was measured in hamsters. The authors observed that depth filtration in the presence of filter aids and nanofiltration removed PrP^{res} reactivity and transmissable spongiform encephalopathy (TSE) infectivity.

CLINICAL TRIALS IN PRIMARY IMMUNODEFICIENCY

In the United States, clinical trials in subjects with primary immunodeficiency have become increasingly standardized.[55] The Food and Drug Administration (FDA) recommends that studies measure the rate of serious bacterial infections during regular infusions of investigational IVIG for 12 months to avoid seasonal biases. Serious infections are defined as bacteremia/sepsis, bacterial meningitis, osteomyelitis/septic arthritis, bacterial pneumonia, and visceral abscess. Diagnostic criteria are listed. Statistical analysis should demonstrate that the upper 99% one-sided confidence interval for the frequency of acute serious bacterial infections is less than one per subject per year.[55]

Infusional adverse events are now defined as those that occur up to 72 hours following an infusion of test product, regardless of other factors that may impact a possible causal association with product administration. The target for this safety endpoint is an upper 95% one-sided confidence limit of less than 0.40.[55]

Pharmacokinetic (PK) data are to be obtained from at least 20 patients. The analysis should include total IgG and several specific antibodies to derive a plasma concentration-time curve, half-life, area under the curve ($AUC^{0\text{-}t}$; $AUC^{0\text{-}infinity}$), volume of distribution, maximum concentration (Cmax), time from start of infusion to Cmax (Tmax), and elimination rate constants. Serum samples for these antibody measurements should be taken after a washout period of 3 to 5 estimated half-lives (3–5 infusions) investigational IGIV. The FDA also desires that trough IgG and IgG subclass levels be measured monthly.[55]

The results of these policies are illustrated in **Table 3**. The time period for recording infusional or temporally associated adverse events has been extended from 30 minutes to 72 hours postinfusion. Each study has reported the incidence of acute serious bacterial infections and other bacterial infections. Although not shown in **Table 3**, pharmacokinetic studies were also performed for each product. The number of PK subjects ranged from 14[62] to 57[57].

TRENDS IN IVIG MANUFACTURING

As shown in **Table 1**, most IVIG products are still produced by cold ethanol fractionation but are now further purified with anion exchange chromatography (DEAE anion exchangers or an equivalent). Plasma fractionation by cold ethanol fractionation involves precipitating proteins by adjusting pH, salt concentration, temperature, and ethanol concentration. Precipitated proteins are removed from proteins still in solution

Table 3
Recent clinical trials in patients with primary immunodeficiency disorders

Product	Study Duration (Months)	Patients Treated	Dose	Acute Serious Bacterial Infect/subj/y	Other Bacterial Infect/subj/y	Related, Temporally Associated AEs (% of Infusions)	Drug-Related SAEs
Carimune NF Liquid (12%)	6	42	200–800 mg/Kg/21–28 d	0	3.65	21.7%[a]	0
Flebogamma 5%	12	51	300–600 mg/Kg/21–28 d	0.061	NR	8.2%[c]	2
Flebogamma 5% DIF	12	46	300–600 mg/Kg/21–28 d	0.021	1.96	11.8%[c]	0
Gammagard liquid 10%	12	61	300–600 mg/Kg/21–28 d	0	0.07	31.2%[c]	2 (1 patient)
Gamunex 10%	9	73	100–600 mg/Kg/21–28 d	0.07	0.18	5.7%[a]	0
Octagam 5%	12	46	300–600 mg/Kg/21–28 d	0.1	0	5.5%[b]	0
Privigen 10%	12	80	200–888 mg/Kg/21–28 d	0.08	3.55	18.5%[b]	5 (1 subject)
Vivaglobin 16%	15	51	34–352 mg/Kg/wk	0.04	4.4	Local, 49%; systemic, 5.4%	0

Abbreviations: AE, adverse event; infect/subj/y, infections per subject per year NF, nanofiltration; SAE, serious adverse event.
[a] 0–48 h postinfusion.
[b] 0–30 min postinfusion.
[c] 0–72 h postinfusion.
Data from Refs.[53–63]

Table 4
United States IVIG distribution data

Year	Kg	% Increase	Liters of Plasma[a]
1998	15,000	—	4,285,714
2002	23,000	53%	6,571,429
2003	24,900	8%	7,114,286
2004	26,900	8%	7,685,714
2005	28,200	5%	8,057,143
2006	32,400	15%	9,257,143
2007	34,200	6%	9,771,429

[a] Assumes 3.5 g IgG obtained per liter of plasma.

by filtration or centrifugation. The most abundant plasma proteins, IgG and albumin, have vastly different physicochemical properties and are readily separated. However, some IgG and albumin is distributed to other fractions at each precipitation step.

In the classical Cohn-Oncley process, fraction II (IgG) was further purified by at least three additional precipitations with IgG losses at each step. Since IgG production is the driving force behind plasma manufacturing capacity, manufacturers have turned their attention to increasing the amount of purified IgG from plasma. Some manufacturers limit IgG precipitation from plasma to a single cold ethanol precipitation step to produce what Cohn referred to as fraction I+II+III. IgG losses are minimized by using I+II+III (or II+III if fraction I-fibrinogen is precipitated earlier) as the starting material for anion exchange chromatography and the virus inactivation and removal steps that have been incorporated into the process.

The importance of increasing IgG yield is illustrated in **Table 4**. Demand for IVIG has increased 128% in the past decade. Manufacturers have been able to meet demand by acquiring underutilized facilities, expanding existing facilities, building new facilities, and increasing yield. IVIG manufacturing changes have been accompanied by an increase in clinical trials in patients with primary immunodeficiency.

There is also a trend to formulate IVIGs as solutions with a protein concentration of 100 mg/mL (10% solutions) and a low pH that favors product stability (pH 4.3 to 5.0.) The increase in IgG concentration from 5% to 10% reduces infusion time, an important feature for patients with primary immunodeficiency who receive large doses every 21 to 28 days all their life. Ten percent IVIGs at low pH are more stable at low ionic strength and therefore sodium chloride is no longer added. In addition, carbohydrate stabilizers are no longer required. Because their tendency to precipitate at increased ionic strength, products of this type may not be diluted with saline or mixed with other IVIGs that contain sodium chloride.[56–58]

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