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Characterization of antibodies in human immunoglobulin products from different regions worldwide

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ABSTRACT

Aim: The antibody levels against a broad spectrum of pathogens were assessed in commercial intravenous immunoglobulin (IVIG) manufactured from pooled plasma obtained from different global regions.

Methods: Twenty-four IVIG commercial lots from eight manufacturers corresponding to 12 brands were analyzed. The plasma was collected in 10 countries/regions. Depending on each pathogen, antibody levels were measured using specific commercial IgG-specific enzyme immunoassay kits or by cell culture neutralization test and guinea pig skin neutralization test. A principal component analysis was performed.

Results: For polio and diphtheria (reference markers of the US authorities), all IVIGs had relevant titers in accordance with reference levels. IVIGs from Canada, Australia, and the USA were positive for titers against globally distributed pathogens or those under vaccination programs in the developed world (parainfluenza, Epstein–Barr, varicella-zoster, influenza B, parvovirus B19, and measles viruses). IVIG from Taiwan and Hong Kong showed low antibody titers for these pathogens but high titers for *Pseudomonas aeruginosa*. IVIG from India had high titers for pathogens frequently found in developing countries (West Nile, dengue, chikungunya, and hepatitis E viruses and *Streptococcus pneumoniae*). IVIGs from Argentina, Spain, Israel, and Czechia showed intermediate antibody concentrations.

Conclusion: The antibody profile in IVIG was greatly influenced by regional characteristics including climate, vaccination programs, and the prevalence of pathogens in the different countries and regions.

Introduction

Since the first use of serum antibodies against infectious diseases in the late 19th century, (von Behring and Kitasato, 1890) the use of antibodies has largely been supplanted by antimicrobial agents. However, the generalized use of antibiotics has led to an emerging crisis of drug resistance for many pathogens throughout the world. Therefore, the focus for treating infections is shifting to include alternative therapies.

Immunoglobulin (Ig) was used successfully for the first time in the middle of the last century to treat a pediatric case of immunodeficiency (Bruton, 1952). Currently, Ig can be used for the treatment of a variety of infections (Bozzo and Jorquera, 2017). In 1981, concentrates of purified functional immunoglobulin G (IgG) known as intravenous immunoglobulin (IVIG), became commercially available in the USA. Recently, the need for readily available therapies to treat coronavirus disease 2019 (COVID-19) during the pandemic has led investigators to turn their attention back to IVIG as an anti-infective therapy (Cao et al., 2020; Mehta et al., 2020; Xie et al., 2020).

IVIG preparations are obtained from pooled human plasma of a minimum size of 1000 donors up to 100 000 donors (Radosevich and Burnouf, 2010). IVIG products contain specific antibodies, resulting in the neutralization of a wide range of specific antigens including pathogens (Keller and Stiehm, 2000). In addition, IVIG preparations from human pooled plasma take advantage of the polyclonal response of every individual donor. IVIG is indicated as a replacement therapy for patients with primary and secondary immunodeficiency. These conditions are characterized by greater susceptibility to infectious diseases due to quantitative or qualitative antibody deficiencies (Schroeder and Dougherty, 2012). In addition to its use in primary and secondary immune deficiencies, IVIG is increasingly used for the treatment of a large number of autoimmune and inflammatory diseases (Perez et al.,

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2017). IVIG efficacy has been demonstrated widely in clinical trials and clinical practice (Lemieux et al., 2005). Minimum potencies for antibodies against poliovirus (type 1, 2, or 3), measles, and diphtheria are generally accepted as reference markers for IVIG (US Pharmacopoeia, 2017; US Food and Drug Administration, 2020).

Depending on the geographical area where the donor population lives, differences in antibody profiles in manufactured IVIG have been reported (Rabel et al., 2012; Goldacker et al., 2014; Planitzer et al., 2011a; Audet et al., 2006; Weeke-Luttmann et al., 1984). For instance, the immune status of plasma donors, influenced by community vaccination programs and pathogen exposure, may be responsible for the antibody level variability in IgG preparations from different geographical areas. Commercial IVIG manufacturing usually implies the fractionation of large plasma pools from healthy donors. This provides a unique tool to investigate the profile of IgG reactivity in these populations. However, there is a lack of systematic studies correlating levels of pathogen-specific antibodies with donor geographic origin.

To further increase the understanding of the characteristics of IVIG antibody profiles, this study determined antibody levels against a broad spectrum of viral and bacterial pathogens in commercial IVIG lots obtained from different geographical areas worldwide.

Materials and methods

Objective

The aim of this study was to characterize antibodies against a range of pathogens in IVIG samples prepared from pooled plasma obtained in different geographical areas worldwide. These IVIG samples represented countries and populations with diverse vaccination profiles and/or natural exposure to different infectious agents.

Study design

Twenty-four IVIG commercial lots from eight manufacturers (identified in this study as A through H) corresponding to 12 brands (identified as A.1 through H.4) were analyzed (see Table 1). The geographic origin of the 24 IVIG lots included North and South America, Europe and Middle East, and Asia-Pacific regions. More precisely, the 10 different countries and territories (at least three for each geographical region) where source plasma was obtained were Argentina, Australia, Taiwan, Hong Kong, India, Israel, Spain, Czech Republic (Czechia), Unites States of America (USA), and Canada. Details of the product and lot distribution by geographical area are shown in Table 1.

Samples from each IVIG lot were analyzed to assess the specific antibody levels against the following viral (n = 19) and bacterial (n = 5) pathogens: chikungunya virus (CHIKV), West Nile virus (WNV), dengue virus (DENV), cytomegalovirus (CMV), echovirus (ECHV), Epstein–Barr virus (EBV), hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis E virus (HEV), influenza A virus (IAV), influenza B virus (IBV), rubella virus (RUBV), mumps virus (MUMV), measles virus (MSLV), parainfluenza virus (PIV), parvovirus B19 (B19), poliovirus type I (PV1), respiratory syncytial virus (RSV), varicella-zoster virus (VZV), *Haemophilus influenzae* type b, *Clostridium tetani*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Corynebacterium diphtheriae* toxin.

Laboratory determinations

The commercial IgG-specific enzyme immunoassay (ELISA) kits used were as follows: Serion ELISA Classic (Serion Immunologics GmbH, Würzburg, Germany) for ECHV, EBV, IAV, IBV, MUMV, PIV, RSV, VZV, and RUBV; Euroimmun ELISA (Euroimmun AG, Lübeck, Germany) for WNV, CHIKV, HEV, and DENV; DiaSorin ELISA (DiaSorin S.p.A., Saluggia, Italy) for HAV; Biokit BioELISA (Biokit S. A., Barcelona, Spain) for HBV and CMV; Virotech ELISA (Virotech Diagnostics GmbH, Rüsselsheim am Main, Germany) for *C. tetani* (detection of IgG antibodies against the tetanus toxoid); SeraQuest ELISA (Awareness Technology, Inc., Palm City, FL, USA) for MSLV; Biotrin ELISA (DiaSorin S.p.A., Saluggia, Italy) for B19; The Binding Site ELISA (The Binding Site Group Ltd, Birmingham, UK) for *H. influenzae* type b; and Mediadiagnost ELISA (Mediadiagnost, Reutlingen, Germany) for *S. pneumoniae* and *P. aeruginosa* (against exotoxin A (ExoA) and elastase (ELA) proteins).

For PV1 and *C. diphtheriae* toxin, antibody titers were assessed using cell culture neutralization and guinea pig skin neutralization tests, respectively. Although opsonization tests are more difficult to perform and have more variability than conventional binding assays such as ELISA, they provide the best function correlate of protection in assessing vaccine immunogenicity.

For the PV1 serum neutralization test, a constant amount of poliovirus was tested against a series of dilutions of the IVIG sample. The EP_{50} (end point 50; i.e., the dilution for which half of the cultures are infected) was determined in those dilutions that showed cytopathic effects when inoculated into cell cultures. A reference preparation was assayed in parallel to define the antibody titer of the sample in comparison to the titer of the reference.

The functional assay to determine antibody titers for diphtheria antitoxin in IVIG was a seroneutralization test in guinea pig skin. A constant amount of previously quantified diphtheria toxin was added to a standard diphtheria antitoxin in a series of dilutions of

Table 1
IVIG products analyzed.

Manufacturer ID	Brand ID	Ig concentration	Origin of plasma	Number of lots
A	A.1	5%	Argentina	3
B	B.1	6%	Taiwan	2
	B.2	6%	Australia	2
C	C.1	5%	India	1
D	D.1	5%	India	1
E	E.1	5%	India	1
F	F.1	6%	Hong Kong	1
G	G.1	5%	Israel	1
H	H.1	5%	Spain	2
		5%	Czechia	2
		5%	USA	2
	H.2	10%	USA	2
	H.3	10%	USA	2
	H.4	10%	Canada	2

IVIG, intravenous immunoglobulin.

the IVIG. These mixtures are inoculated intradermally into guinea pigs. Potency was calculated by determining the doses of gamma globulin necessary to protect the guinea pigs from the cutaneous effects of diphtheria toxin.

Results were expressed preferentially as international units (IU) per gram of Ig content of the sample. Otherwise results were expressed as units (U), relative units (RU), antitoxin units (AU), and Paul Ehrlich Institut units (PEIU) per gram of Ig. For PV1, results were expressed as the ratio sample/Center for Biologics Evaluation and Research (CBER) lot 176. For *P. aeruginosa*, results were expressed as the ratio antibody titer/borderline titer limit.

Additionally, an immunonephelometric assay was performed to measure the levels of residual immunoglobulin M (IgM) (Jorquera, 2009) in IVIG lots. This could determine possible IgM interactions that could interfere in the interpretation of IgG results. Results were expressed in grams per liter (g/l) per product brand (representative of the manufacturing process).

Calculations and data analysis

All titer analyses were performed in duplicate. The IVIG antibody titer (mean ± standard deviation (SD)) against each pathogen was calculated for each geographical plasma source.

Pathogens were grouped as follows. The first group comprised those accepted as reference markers according to US regulations (US Pharmacopoeia, 2017). Specifications were set at ≥0.17 ratio sample/CBER reference at 10% IVIG concentration for PV1 and ≥1.21 AU/ml for *C. diphtheriae* at 10% IVIG concentration (US Food and Drug Administration, 2020). Reference values were not specified for MSLV because the titer was determined by ELISA method.

The other pathogens were grouped according to their more relevant mode of transmission (vector-borne, water/food-borne, airborne, droplet contact, body fluids contact, and nosocomial/opportunistic). Countries were sorted according to their predominant climatic area (tropical vs non-tropical) (Pidwirny, 2006), representing the environment where certain diseases are prevalent, and also by their human development index (HDI) (United Nations Development Programme, 2019), a proxy of the health/sanitary conditions and vaccination coverage.

Principal component analysis (PCA) was performed with the data from the antibody titers obtained for each IVIG sample using IBM SPSS Statistics version 23 (IBM Corp., Armonk, NY, USA).

Software used for calculations and graphs were Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA) and GraphPad Prism v6 (GraphPad Software Inc., San Diego, CA, USA).

Results

Table 2 summarizes the antibody titers of all pathogens in the IVIG preparations from the 10 countries and territories worldwide and geographical/climatic areas and their HDI, as well as the total titer per pathogen (mean ± SD).

IgG and antibody levels for reference pathogens

For PV1, the antibody titer values were highest in Australia, Hong Kong, Israel, Czechia, and Canada (8–10 ratio/g Ig, compared with 3–5 ratio/g Ig for the other regions). Anti-diphtheria antibody results showed a relatively wide range of values among regions. The highest titers were observed in Argentina and Australia (close to >200 AU/g Ig). Titers for IVIG from Taiwan and Czechia were much lower than the rest, averaging 20 ± 0 AU/g Ig for both regions. For MSLV, titer values ranged from 84 IU/g Ig in Hong Kong to 281 ± 15 IU/g Ig and 330 ± 2 IU/g Ig in Canada and Australia, respectively.

IgG and antibody levels for non-reference pathogens

For vector-borne (mosquito) disease pathogens (CHIKV, DENV, and WNV), the IVIG samples obtained from donors in India showed titer values much higher than those of the other IVIG, approximately 25- to 50-fold.

Differences in the geographical distribution were also observed in antibodies against water/food-borne disease pathogens, specifically for HAV and HEV. Although in both cases the highest titers were again detected in India, HAV showed more variability among countries.

Regarding airborne disease pathogens (RSV, PIV, and B19) and pathogens transmitted via droplet contact (IAV, IBV, VZV, MUMV, and RUBV), the distribution of titers among countries was quite homogeneous, except for a tendency for low B19 titers in Asian tropical regions (India, Taiwan, and Hong Kong).

More variability was seen for pathogens transmitted via body fluids contact (CMV, EBV, and HBV). For CMV, a trend towards high titers was observed in the Asia-Pacific region (Taiwan, Hong Kong, and Australia), while for EBV and HBV, the trend to higher titers was observed in countries with a high HDI (Canada, USA, and Australia).

Regarding pathogens causing nosocomial and opportunistic diseases (*P. aeruginosa*, *H. influenzae*, *S. pneumoniae*, and *C. tetani*), a rather irregular distribution of titers was observed for *P. aeruginosa*. For *H. influenzae*, *S. pneumoniae*, and *C. tetani,* the distribution was more homogeneous, except for a peak titer for *H. influenzae* in Israel.

IgG and antibody levels for all pathogens

When analyzed globally, IVIG samples from Australia and Canada showed the highest antibody titers for most of the studied pathogens. More precisely, Australia had the highest titers for MSLV, ECHV, MUMV, IBV, PIV, and CMV, while Canada had the highest titers for RUBV, VZV, B19, EBV, RSV, PV1, and *C. tetani*. Conversely, samples from Hong Kong and Taiwan globally showed the lowest titers. Hong Kong had the lowest titers for RUBV, B19, MSLV, ECHV, IAV, PIV, and *C. tetani*, while Taiwan had the lowest titers for DENV, VZV, IBV, PV1, and *S. pneumoniae*.

Principal component analysis

PCA scores based on the pathogen titers obtained from the different IVIG samples are shown in Figure 1. The two principal components accounted for 58.80% of total variation in antibody titers. Along the component 1 (PCA1) axis, the IVIGs from Canada, Australia, and the USA were positively correlated with pathogens globally distributed or under vaccination programs in the developed world (i.e., PIV, EBV, VZV, IBV, B19, MSLV) and were separated from Taiwan and Hong Kong, which showed high *P. aeruginosa* antibody titers and low titers of PIV, EBV, VZV, IBV, B19, and MSLV antibodies. On the component 2 (PCA2) axis, IVIG from India, which was correlated to pathogens frequent in developing countries (WNV, DENV, CHIKV, HEV, *S. pneumoniae*), was separated from the rest of the IVIGs. The PCA also clustered together the IVIGs from Argentina, Spain, Israel, and Czechia in the center of the plot, showing intermediate antibody concentrations (Figure 1).

IgM levels in IVIG brands

With the exception of two IVIG brands (C.1 and D.1), for which IgM titers were 1.266 g IgM/l and 1.243 g IgM/l, respectively, most IVIG brands showed very low or undetectable IgM concentrations. These values ranged from <0.002 g IgM/l in brands H.1 and E.1 to

Table 2
Antibody titers of all pathogens in IVIG preparations from the 10 countries studied, distributed according to their climatic area (Pidwirny, 2006) and human development index (HDI) (United Nations Development Programme, 2019)

Pathogen		Country/region with geographical/climatic area, human development index (HDI), and antibody titer (mean ± standard deviation)										
Markers/ mode of transmission	Name	Titer, units per g Ig	Tropical India (*N* = 3)	Taiwan (*N* = 2)	Hong Kong (*N* = 1)	Non-tropical Argentina (*N* = 3)	Czechia (*N* = 2)	Spain (*N* = 2)	Israel (*N* = 1)	Canada (*N* = 2)	USA (*N* = 6)	Australia (*N* = 2)	Total (*N* = 24)
			HDI = 0.65	HDI = 0.88	HDI = 0.94	HDI = 0.83	HDI = 0.89	HDI = 0.89	HDI = 0.91	HDI = 0.92	HDI = 0.92	HDI = 0.94	
Reference markers	Measles virus	IU	160 ± 17	106 ± 4	84	144 ± 8	110 ± 12	168 ± 4	104	281 ± 15	129 ± 28	330 ± 2	161 ± 72
	C. diphtheriae	AU	90 ± 61	20 ± 0	140	197 ± 64	20 ± 0	70 ± 28	90	145 ± 7	157 ± 56	260 ± 0	128 ± 80
	Poliovirus	Ratio 1	3 ± 1	3 ± 0	7	4 ± 1	8 ± 0	4 ± 0	8	10 ± 0	5 ± 1	8 ± 0	6 ± 2
Vector-borne	Dengue virus	RU	27 373 ± 3709	222 ± 13	258	661 ± 57	374 ± 12	257 ± 86	527	484 ± 159	540 ± 208	787 ± 149	3852 ± 9149
	Chikungunya virus	RU	26 513 ± 5290	180 ± 18	260	104 ± 6	169 ± 10	159 ± 1	138	347 ± 24	229 ± 26	1147 ± 79	3568 ± 8999
	West Nile virus	RU	12 948 ± 699	518 ± 11	425	856 ± 71	664 ± 86	375 ± 21	1542	639 ± 88	1323 ± 242	867 ± 58	2394 ± 4097
Water/food-borne	Hepatitis A virus	IU	1356 ± 150	271 ± 3	209	998 ± 44	124 ± 30	844 ± 96	626	407 ± 25	290 ± 59	510 ± 42	581 ± 410
	Hepatitis E virus	IU	165 ± 52	21 ± 1	37	30 ± 3	41 ± 2	43 ± 10	18	33 ± 1	24 ± 2	20 ± 1	46 ± 49
	Echovirus	U	600 ± 53	627 ± 47	491	735 ± 26	639 ± 33	801 ± 100	908	708 ± 19	887 ± 172	983 ± 1	760 ± 163
Airborne	Respiratory syncytial virus	U	689 ± 82	436 ± 24	437	524 ± 25	557 ± 17	603 ± 21	414	773 ± 11	648 ± 69	704 ± 62	605 ± 114
	Parainfluenza virus	U	1465 ± 52	1293 ± 79	1076	1418 ± 74	1304 ± 3	1529 ± 27	1304	1822 ± 15	1720 ± 113	2103 ± 97	1561 ± 267
	Parvovirus B19	IU	1134 ± 231	624 ± 7	616	3145 ± 77	2261 ± 188	2030 ± 35	2372	4113 ± 292	2820 ± 329	2061 ± 62	2288 ± 1020
Droplet contact	Influenza A virus	U	627 ± 55	495 ± 38	463	622 ± 46	533 ± 10	664 ± 21	649	775 ± 13	893 ± 66	643 ± 9	689 ± 149
	Influenza B virus	U	693 ± 4	495 ± 41	601	601 ± 17	546 ± 24	629 ± 13	743	786 ± 32	866 ± 79	868 ± 7	711 ± 140
	Mumps virus	U	37 250 ± 2070	44 030 ± 8800	47 839	30 403 ± 605	29 027 ± 1192	31 860 ± 1896	39 425	54 671 ± 1407	31 665 ± 5655	57 102 ± 1361	38 142 ± 10 135
	Rubella virus	IU	8305 ± 617	6124 ± 299	4584	8697 ± 160	10 187 ± 887	8296 ± 260	5530	10 515 ± 302	6295 ± 608	10 041 ± 78	7884 ± 1842
	Varicella-zoster virus	IU	90 ± 21	77 ± 2	102	97 ± 7	146 ± 1	145 ± 3	103	172 ± 4	171 ± 20	145 ± 1	132 ± 38
Body fluids contact	Cytomegalovirus	PEIU	366 ± 19	803 ± 171	777	429 ± 16	250 ± 37	320 ± 0	220	542 ± 22	389 ± 112	975 ± 105	478 ± 232
	Epstein–Barr virus	U	11 257 ± 603	11 385 ± 17	11 534	16 409 ± 933	15 354 ± 1507	18 171 ± 649	8530	24 216 ± 535	22 596 ± 4393	20 516 ± 3837	17 414 ± 5524
	Hepatitis B virus	IU	16 ± 3	29 ± 1	42	34 ± 3	36 ± 4	32 ± 2	85	72 ± 4	60 ± 7	61 ± 1	46 ± 20
Nosocomial/ opportunistic	*C. tetani*	IU	281 ± 20	238 ± 42	220	214 ± 11	245 ± 21	200 ± 0	273	342 ± 265	304 ± 30	255 ± 7	265 ± 47
	H. influenzae	mg	0.31 ± 0.07	0.29 ± 0.01	0.22	0.27 ± 0.01	0.31 ± 0.01	0.31 ± 0	0.71	0.27 ± 0.02	0.23 ± 0.02	0.33 ± 0.03	0.3 ± 0.1
	P. aeruginosa (ELA)	Ratio 2	165 ± 19	826 ± 196	549	86 ± 7	58 ± 2	73 ± 4	82	557 ± 30	223 ± 248	703 ± 122	298 ± 290
	P. aeruginosa (ExoA)	Ratio 2	615 ± 49	795 ± 33	668	254 ± 10	108 ± 2	246 ± 37	228	343 ± 6	247 ± 152	490 ± 24	373 ± 219
	S. pneumoniae	mg	11 ± 2	7 ± 1	7	7 ± 1	7 ± 1	9 ± 1	9	9 ± 0	8 ± 1	8 ± 0	8 ± 1

AU, antitoxin units; *C. diphtheriae*, *Corynebacterium diphtheriae*; *C. tetani*, *Clostridium tetani*; ELA, elastase; ExoA, exotoxin A; *H. influenzae*, *Haemophilus influenzae*; Ig, immunoglobulin; IVIG, intravenous immunoglobulin; IU, international units; *P. aeruginosa*, *Pseudomonas aeruginosa*; PEIU, Paul Ehrlich Institut units; Ratio 1, sample/CBER lot 176; Ratio 2, antibody titer/borderline titer limit; RU, relative units; *S. pneumoniae*, *Streptococcus pneumoniae*; U, units.

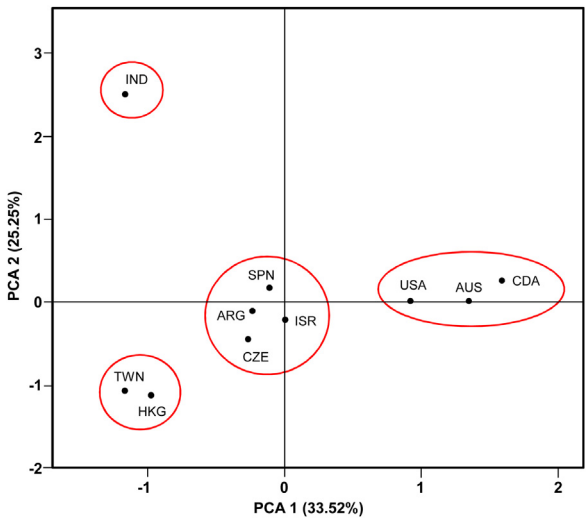

Figure 1. Principal component analysis (PCA) plot of the first two components, showing a clear separation of four groups of intravenous immunoglobulin preparations based on their antibody titers. Percentages of variation explained by each component are indicated along the axes. Argentina (ARG), Australia (AUS), Taiwan (TWN), Hong Kong (HKG), India (IND), Israel (ISR), Spain (SPN), Czechia (CZE), United States of America (USA), and Canada (CDA).

0.087 ± 0.032 g IgM/l in brand H.4. Details of all brands are shown in Table 3.

Discussion

Generally, the basal antibody reactivity in a population defines, to some extent, the primary community barrier against pathogen expansion. The antibody profile of a given population responds primarily to two factors: the incidence of the pathogens in that geographical region and the specific vaccination programs in their communities. However, in the case of opportunistic or universally distributed pathogens, these factors may not apply. Recently, with the COVID-19 pandemic, we have learned how quickly emergent pathogens can spread worldwide (Lu et al., 2020). In any case, the composition of an IVIG product is representative of the antibody profile of the donor population, and thus, a close representation of the immunoreactivity against pathogens in the community (Keller and Stiehm, 2000; Díez et al., 2020). In addition, in contrast with other immunoglobulins, IgG is representative of a stable immunological response, probably the result of repeat exposure to the pathogen agent or its vaccine (Keller and Stiehm, 2000; Vani et al., 2008).

Large fractionation pools used in IVIG manufacturing offer a unique tool to conduct a prospective analysis of the immunoreactivity against pathogens of a given population. In general, there is a lack of information on the titers for most antibodies in IVIG products. The present study appears to be the most extensive study to date covering the largest number of pathogen-specific antibodies (representative of diseases affecting diverse human populations) and IVIG lots selected on a worldwide basis. The antibody composition of IVIGs obtained from donors from different geographical areas was characterized and also their predominant climatic area (Pidwirny, 2006) and their standard of living were considered (United Nations Development Programme, 2019).

Nowadays, antibody titers against PV1, MSLV, and *C. diphtheriae* are required for IVIG batch release (US Pharmacopoeia, 2017; Jorquera, 2009). Otherwise the evaluation must rely on the published literature, when available (WNV, (Rabel et al., 2011) CMV, (Miescher et al., 2015; Planitzer et al., 2011b; Shibaguchi et al., 2010) ECHV,(Planitzer et al., 2011a) HAV, (Farcet et al., 2010; Wu et al., 2013) HBV, (Wu et al., 2013) IAV–IAB, (Weeke-Luttmann et al., 1984; Hong et al., 2011; Kubota-Koketsu et al., 2012; Sullivan et al., 2009; Tian et al., 2016) RUBV, (Weeke-Luttmann et al., 1984; Wu et al., 2013) MSLV, (Audet et al., 2006; Weeke-Luttmann et al., 1984; Wu et al., 2013; Nobre et al., 2014) RSV, (Tian et al., 2016) VZV, (Wu et al., 2013; Nobre et al., 2014; Maranich and Rajnik, 2009) *H. influenzae*, (Mikolajczyk et al., 2004) *C. tetani*, (Wu et al., 2013; Nobre et al., 2014) *S. pneumoniae*, (Mikolajczyk et al., 2004) *P. aeruginosa*, (Nakae et al., 1994) *C. diphtheria* (Wu et al., 2013; Nobre et al., 2014).

The study results for PV1 and *C. diphtheriae* showed that all IVIGs were in agreement with the antibody titers stated by US authorities (US Pharmacopoeia, 2017; Jorquera, 2009). For MSLV, the methodology used was appropriate to assess the antibody levels of this pathogen, although it limited the interpretation of the results with respect to reference values. Nevertheless, it is important to clarify that above a minimum that currently has only been set for these three pathogens, the antibody titer should not be regarded as an indicator of IVIG efficacy.

Globally, the IVIG samples analyzed showed significant levels of antibodies against most of the studied pathogens, although differences associated with the geographic origin of the pooled plasma were observed. This confirms the existence of unique properties in the plasma source of the IVIGs (Rabel et al., 2012; Goldacker et al., 2014; Planitzer et al., 2011a; Audet et al., 2006; Weeke-Luttmann et al., 1984, Nobre et al., 2014). Hence, IVIG lots from Australia and Canada showed the highest titers for 13 of the 24 pathogens studied, most of them including globally distributed viruses (e.g., IBV, PIV, B19, and EBV) (Pison, 2019), and viruses influenced by the local vaccination programs (e.g., MSLV, RUBV, PV1, and MUMV (Young et al., 2017a; Young et al., 2017b; Gidding

Table 3
Amount of IgM in the analyzed IVIG brands

Brand ID	Ig concentration	Origin of plasma	Number of lots	IgM (g/l) Mean ± SD
A.1	5%	Argentina	3	0.072 ± 0.026
B.1	6%	Taiwan	2	<0.003
B.2	6%	Australia	2	<0.003
C.1	5%	India	1	1.266
D.1	5%	India	1	1.243
E.1	5%	India	1	<0.002
F.1	6%	Hong Kong	1	<0.003
G.1	5%	Israel	1	<0.01
H.1	5%	Spain, Czechia, USA	6	<0.002
H.2	10%	USA	2	<0.004
H.3	10%	USA	2	<0.011
H.4	10%	Canada	2	0.087 ± 0.032

Ig, immunoglobulin; IgM, immunoglobulin M; IVIG, intravenous immunoglobulin; SD, standard deviation.

et al., 2005; Macartney et al., 2017; Farcet et al., 2019). Australia and Canada, together with the USA, include communities with the highest standards of living on our list, (United Nations Development Programme, 2019) and IVIG lots from these countries showed high titers against PIV, IBV, and EBV. By contrast, Taiwan and Hong Kong lots showed lower titers against most of these pathogens. Interestingly, plasma from Taiwanese subjects has been reported to have low titers against MSLV, RUBV, HAV, HBV, and VZV (Wu et al., 2013). This has been ascribed to immune memory decline or loss.

A decreased HAV antibody seroprevalence in Europe and the USA has also been described, (Farcet et al., 2010) which is in accordance with the present study results. Conversely, high titers against different subtypes of IAV (H5N1,(Sullivan et al., 2009) H2N2, (Kubota-Koketsu et al., 2012) and H1N1, (Hong et al., 2011)) and VZV (Maranich and Rajnik, 2009) have been found in IVIG preparations from the USA. In the present study, IVIG samples from the USA, together with Canada, showed the highest titers against IAV and VZV. While lots from Argentina, Spain, Israel, and Czechia globally showed intermediate antibody concentrations, high antibody levels against pathogens transmitted by mosquitoes (DENV, CHIKV, and WNV) and pathogens related to suboptimal access to good quality drinking water or contaminated food (HAV and HEV) were observed in lots from India. The developing country status of India (United Nations Development Programme, 2019), together with its tropical climate create conditions favorable to the pathogen vector in that country, might explain these results.

PCA confirmed the existence of a geographical distribution, as suggested by the antibody titer study. The component of pathogens of global distribution or under vaccination programs discriminated three groups of regions in agreement with their development and income levels (United Nations Development Programme, 2019; Hajizadeh, 2018). On the other hand, the component of water/food-borne, vector-borne, or children-affecting pathogens clearly differentiated the samples from India, a developing country in a mostly tropical environment. DENV, CHKV, and WNV are endemic in India; there is no vaccine for these viruses and they could cause significant outbreaks in other parts of the world. The increasing global mobility of human populations is creating challenges for epidemiology and disease control (Pybus et al., 2015; Anukumar et al., 2014; Leshem et al., 2012).

The diversity in the strategy of IgG purification among manufacturers could be associated with small variations in the content of IgM in these IVIG products. IgM might be able to interfere with the ELISA kits used for IgG determination. All IVIG preparations showed very low or undetectable IgM concentrations, with the exception of some IVIG lots from manufacturers C and D. However, the elevated antibody titers observed against HEV, WNV, CHIKV, and DENV indicated that the possible presence of IgM did not interfere in the IgG measurements conducted in this study.

In conclusion, we have presented an extensive study of the specific characteristics of the antibody profiles in IVIG that arise as a result of the different geographic origins of the donor population. All IVIG products are, by definition, effective in clinical practice and all of them have met the required levels for polio and diphtheria antibodies stated by authorities. On the other hand, the antibody profiles were greatly influenced by regional characteristics, including vaccination programs and the prevalence of pathogens in the different regions. Frequent IVIG antibody profiling could be useful in the future to better understand pathogen exposure and immunological barriers in a given population. More research is also needed to understand the potential benefit of expanding vaccination programs to plasma donors from each region or country.

Ethics statement

Not applicable.

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Conflict of interest

The authors are full-time employees of Grifols, a manufacturer of intravenous immunoglobulin products and the funder of the study.

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