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RESEARCH ARTICLE

A snapshot of ABO, RH, and JK blood group systems in modern Ireland

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Abstract

Objectives: This study aimed to capture a snapshot of the Irish population to determine if there had been any changes in the ABO and RH blood group system (BGS) distribution from previous Irish studies and to establish an Irish JK BGS frequency, providing real time donor information to the Irish Blood Transfusion Service (IBTS).

Background: Ireland's population is constantly increasing and becoming more diverse, this has potential implications for the IBTS to provide blood with extended phenotypes for certain cohorts of patients.

Materials and methods: All first time blood donors had relevant testing performed in the Automated Donor Grouping (ADG) laboratory using the Beckman Coulter PK7300 analyzer with appropriate antisera by validated methods. All pertinent information and test results were categorized and analyzed.

Results: The number of donors tested was 3427. ABO phenotype: A: 29.82%, B: 12.02%, O: 54.95% and A,B: 3.21%. RHD: 82.26%. RHCE: R_1R_1: 17.62%, R_2R_2: 2.89%, R_1R_2: 13.95%, R_1r: 33.35%, R_2r: 13.07%, R_or: 1.25%, R_1R_Z: 0.06%, R_2R_Z: 0.06%, r′r: 0.55%, r″r: 0.53%, rr: 16.66%. Kidd phenotype: Jk(a + b+): 49.63%, Jk(a-b+): 23.34%, Jk(a + b-): 27.02%.

Conclusion: The observed frequencies for the relevant BGSs remained relatively unchanged to the prevalence values expected; however, statistically significant differences between the 2015 study and some of the previous studies were found for ABO distribution. 14.24% of the first time donors were born outside Ireland and statistically significant differences (P-value < 0.001) were noted for aspects of the ABO and Rh phenotype distribution for the Irish born donors (BiI) vs those born outside Ireland (BoI).

KEYWORDS
ABO, Ireland, JK Irish, Kidd, RH

1 | INTRODUCTION

The Irish Blood Transfusion Service (IBTS) is a blood establishment whose functions are set out in Statutory Instruments and European directives. The Automated Donor Grouping Laboratory (ADG) tests all blood donations in the Irish Republic. An ABO, RHD, RHCE, K antigen, antibody and a high titer antibody screen are determined for each donation. Extended antigen typing is undertaken to meet the

requirements of patients with alloantibodies or transfusion dependent patients. Rare and complex extended phenotypes can be difficult to provide.

Worldwide, blood group system (BGS) distribution varies among populations.[1,2] Generally, Group A has the greatest frequency in North-western Europe and Group B in parts of Southeast Asia.[1,2] Up to 77% of Group A frequencies are found in Aboriginal people in South Australia.[2] Populations with Group O greater than 60% are found in native people in the Americas and some parts of Africa and Australia.[1,2] Native people in South and Central America were almost 100% Group O before European arrival.[2]

BGS distribution in Ireland was first examined in 1937 and 1940 where the authors found that Group O frequency was higher in Dublin than in other parts of Europe.[3,4] In 1947, it was found that Group O along the western seaboard of Ireland reached 60% whereas the east coast had a higher frequency of Group A and less Group O.[5] One assumption was that successive invasions had pushed the native Irish populations further west.[5]

Ireland's population has been influenced by invasions, migration and settlement. [5,6] From 1846 to 1848, the population fell from eight million to six million during the potato famine.[6] This decline continued; in the 1950's, Irelands population was 2 898 264[7] which, is the approximate period of time when the previous studies were undertaken. Ireland's population is constantly increasing; census data indicates a population increase to 4 761 865 million in 2016[8] from 4 588 252 million in 2011.[9]

According to the 2016 census, 535 475 of people registered in Ireland were non-Irish nationals[10] who came from over 200 nations, 12 of these nations accounted for 73.6% of Irelands non-Irish population.[11] The proportion of the population that identified as "white Irish" was 82.2%.[12] This increasingly ethnically diverse society has potential implications for the IBTS in its requirements to provide blood products. To provide Jk(a-b-) blood to a patient with anti- Jk3 is extremely difficult, if not impossible to obtain from an ethnically Irish donor population.[13]

The expected prevalence values determined for the Irish population for this study were Group A: 31%, Group B: 11%, Group O: 56% and Group A,B: 3%[14,15] with RhD antigen positive: 83%.[14-16] A previous Irish Rh phenotype profile, indicated higher R1r's (DCe/dce) and R2r's (DcE/dce) with lower rr's (dce/dce) in western Ireland.[17] The expected JK BGS phenotype prevalence for a Caucasian population were: Jk(a+b+): 50.3%, Jk(a−b+): 23.4%, Jk(a+b−): 26.3%.[13]

The objectives of this cross sectional study were to test and categorize first time donors during a specific time period, to determine if there had been any statistically significant changes in the Irish population's ABO BGS and RhD antigen frequency relevant to the previous studies and in relation to the distribution of these BGS's within Ireland; to determine an RHCE and JK frequency; to assess if there were differences of the BGS's distribution within the two resultant populations, that is, born in Ireland (BiI) vs born outside Ireland (BoI) donors. A corollary was the participation of donors from different countries in blood donation in Ireland compared with their proportions in the Irish population. The outcome would be a snapshot of ABO (001), RHD (004), RHCE (004), and JK (009) BGS prevalence in Ireland.

2 | MATERIAL AND METHODS

2.1 | Subjects studied

Donors donating for the first time to the IBTS were included in this study as this avoided any bias that would occur if using known donors from clinic call-ups. Donors from all 26 counties were represented. Clinic locations were dependent on IBTS clinic rosters. Testing was performed over a 14 week period from August to December 2015. Venous blood was collected in Vacuette K3 EDTA anticoagulant, refrigerated overnight and on arrival to the laboratory centrifuged prior to testing at 3500 rpm (2739 g) for 5 minutes. All procedures were undertaken according to ADG standard operating procedures (SOP's). The final sample size was 3427 with 2939 donors BiI and 488 donors BoI. The Kidd profile had 3423 donors, (2935 BiI/488 BoI) with a loss of four donors (BiI) due to positive infectious disease markers. Both ABO profiles and Rh phenotype testing had been completed prior to this notification.

2.2 | Ethical considerations

Ethical approval was obtained from the IBTS in accordance with research ethics and governance policy and procedures, including code of practice for professional integrity in the conduct of research and the Ulster of University Ethics Filter Committee. Informed consent was included with the Health and Lifestyle Questionnaire (HLQ) completed by all donors. The donors were informed that parts of, or the entire donation may be used for purposes other than direct transfusion to a patient; uses such as research and development were given. A project number was assigned to each donor to fulfill all ethical requirements in relation to confidentiality of the donor.

2.3 | Instrument 1

The Beckman Coulter PK 7300 automated system was the platform used, which when combined with appropriate reagents facilitated hemagglutination reactions in Beckman Coulter microtiter plates.[18] The PK system interfaced to the laboratory information management system (LIMS) ePROGESA via electronic document management system (eDMS).[19]

2.4 | Instrument 2

Hook, Tucker, and Zenyz (HTZ) Qasar IV[20] facilitated serological testing using BioRad gel card techniques. Confirmation of some ABO weaker reverse groups using BioRad ID-card Diaclon ABO/D + reverse grouping was performed with BioRad ID-DiaCell A_1 and B.

2.5 | Test

Blood groups were determined by the presence or absence of agglutination when the test red blood cells (RBCs) were reacted against specific antisera. The ABO/RHD determination for each donation was performed on two separate ABO profiles. An RHCE and JK phenotype were determined, each with its own profile on the PK7300's.

2.6 | Antisera

The antisera for the PK7300 microplate technique had been validated in ADG to ensure potency and specificity without a compromise in sensitivity.[21]

The first ABO profile (ABOa) used the following clones; anti-A [Bioscot-millipore; Birma-1], anti- B [Bioscot-millipore; LB-2], anti-A,B [Bioscot-millipore; ES-15/ES-4], anti- D 1 [Diagast -Totem; p3x61 + p3x21223B10 + p3x290 + p3x35], and anti- D 2 [Immucor -Novaclone; D415/D175].

The second ABO profile (ABOb) used anti -A [Immucor-Novaclone; A98], anti- B [Immucor- Novaclone; B84 + B97], anti -A,B [Immucor- Novaclone; A98 + B84 + B97 + AB125], anti- D 1 [Bioscot-millipore; Rum-1], and anti -D 2 [Bioscot-millipore; MS-201].

The two anti- D antisera on ABOa detected DVI positive RBCs; whereas the two anti- D antisera on ABOb did not detect DVI positive RBCs.

The Rh phenotype profile used the following antisera clones; anti-C 1 [Bioscot-millipore; MS 24] & anti -C 2 [Imumed- antitoxin; MS273], anti- c 1 [Bioscot-millipore; MS33] & anti- c 2 [Imumed- antitoxin; MS35], anti- E 1 [Bioscot-millipore; MS80/MS258] & anti -E 2 [Imumed antitoxin;MS258/906], anti- e 1 [Bioscot-millipore; MS16/21/63] & anti -e 2 [Imumed- antitoxin; MS16/21/63]. A further anti -D antisera [Imumed- antitoxin; MS26/TH28] was necessary for the interpretation of the Rh phenotype (most probable Rh genotype).

The JK phenotype profile used anti -Jk^a 1 [Bioscot-millipore; MS15] & anti- Jk^a 2 [Imumed-antitoxin; MS15], anti -Jk^b 1 [Bioscot-millipore; MS 8] & anti -Jk^b 2 [Imumed- antitoxin; MS 8].

2.7 | Reagents

2.7.1 | Diluents

Phosphate buffered serology saline (PBSS) pH 7.0 [Biosciences] was used for dilution to improve reaction patterns.[22] RBC typing and antibody screening was performed by an enzyme technique[22] using Bromelain [Sigma-Aldrich], a proteolytic enzyme used daily at a 0.1% working solution.

2.7.2 | Reagent RBCs

The five reagent RBCs (rRBCs) required were prepared from RBC packs with known phenotypes on a daily basis. RBCs were washed and prepared in saline suspension at concentrations of 1.25%-1.45%, depending on the validated RBC concentration required for the relevant profile on the PK7300's.

An O R_1R_1 K+/K- and an O R_2R_2 K+/K- rRBC were used for antibody screening and an A_1B rRBC was used to detect anti A,B high titer positive donors on the ABOa profile.

The testing of the donor plasma for its hypothetical ABO antibody/ ies (reverse group) was performed using A_1 RhD- and B RhD-rRBCs on the ABOb profile.

2.8 | Controls

An inert monoclonal control [Bioscot- Millipore] was the negative antisera control used with each set of antisera prepared.

Each profile was controlled with the relevant controls placed throughout each run.[21]

ABOa RBC controls were: A_2B, A_1, B, weak RhD+, and DVI+ which were prepared from RBC packs daily, washed and resuspended in saline. An R1r K+ RBC (a previous donation with historical phenotype) was also necessary.

Anti D [Quotient-Albacheck; 0.3 IU/mL] was the sensitivity control for antibody screening. An anti- A,B [Bioscot-millipore; ES-15/ES-4] prepared at 1:16 dilution was used as a control for donor anti- A,B high titer detection.

ABOb used the same controls as ABOa except no requirement for a K+ cell; ABOb profile also had a group A, B, and O (RBC controls selected from a previous ABOb run where strong reverse group reactions were observed) to control the reverse ABO.

The Rh phenotype profile had the following controls: R_1R_1, R_2R_2, R_1R_2, R_1r, R_2r, R_or, r′r, r″r and rr, where using Fisher-Race terminology; R_1 = DCe, R_2 = DcE, R_o = Dce, R_z = DCE, r′ = dCe, r″ = dcE, and r = dce.[13]

The JK profile was controlled with: two Jk(a−b+), two Jk(a+b−), and two Jk(a+b+) controls. RBC controls for the Rh phenotype and Kidd profiles were selected from previous testing / historical donor phenotypes.

2.9 | Quantitative variables

Once testing was complete, the ABO, RhD, Rh phenotype, and JK phenotype results were recorded, together with relevant donor demographic data. The BGS's by donor were further categorized by BiI/BoI and county/country. The BoI donors were classified using United Nations country and area codes.[23]

The outcome measure was the blood group antigen presence or not on the donor RBCs. The result was Group A, B, O or A,B and depending on D antigen presence or not, each donors blood group was further defined to A RhD positive, A RhD negative, B RhD positive, B RhD negative, O RhD positive, O RhD negative, AB RhD positive, or AB RhD negative. The Rh phenotype frequency outcomes (most probable genotype) were R_1R_1, R_2R_2, R_1R_2, R_1r, R_2r, R_or, R_1R_z,

R_2R_Z, r′r, r″r, or rr. The outcome measures for JK phenotypes were: Jk(a+b+), Jk(a−b+), or Jk (a+b−). All outcomes were independent of each other and from the categorical data, all relevant frequencies were calculated.

2.10 | Statistics

The expected prevalence (p) of each BGS was determined from previous Irish studies for A, B, O, A,B[14,15] and the D antigen[14-16] and from that expected for a Caucasian population for the Jk antigens (Jk^a and Jk^b).[13] These expected prevalence values (p) were A: 31%, B: 11%, A,B: 3%, O: 56%,[14,15] D; 83%[14-16] and the expected JK phenotype prevalence's were: Jk(a+b+): 50.3%, Jk(a−b+): 23.4%, Jk(a+b−): 26.3%.[13] The difference (d) that would be clinically relevant for this study[24] was determined to be 5.0% for all groups except 3.0% for Group B and 1.0% for group A,B. Power calculations were performed at 80% and 90% power. [24,25] Formula for 80% power was: (16 [s^2/d^2]) and 90% power: (21[s^2/d^2])[26] where: $s^2 = (p\ [1-p])$. Confidence limits at 95% were estimated, assuming the binomial distribution conformed to a normal distribution using (p + / −1.96 * SE) where 1.96 was the Z value[25] and SE calculated as ($\sqrt{}$ p [1−p] / n)[24-26] where n was the sample size determined from the power calculations.

Further assessment for associations between the relevant categorical variables was done using contingency tables and a chi square test with *P*-values calculated.[25] A *P*-value less than 0.05 was considered statistically significant for this study as this indicated evidence of a difference between the variables been analyzed.[25] This analysis was performed using SPSS 22[27] and Stata software.[28]

Allele frequency calculations were performed[29-34] assuming Hardy Weinberg Equilibrium (HWE) rules[34] applied to the population. The Rh phenotype haplotype frequency was estimated from the Rh phenotype frequencies.

3 | RESULTS

3.1 | Total Irish donor population (n = 3427)

The ABO phenotype distribution in modern Ireland was Group A: 29.82%, Group B: 12.02%, Group O: 54.95%, and Group A,B: 3.21%. RhD positive phenotype distribution was 82.26% and RhD negative: 17.74% (rr, r′r, and r″r). This was further refined to: A RhD positive: 24.31%, A RhD negative: 5.52%, B RhD positive: 9.78%, B RhD negative: 2.25%, O RhD positive: 45.55%, O RhD negative: 9.40%, AB RhD positive: 2.63%, and AB RhD negative: 0.58%.

The Rh phenotype frequency for Ireland was: R_1R_1: 17.62%, R_2R_2: 2.89%, R_1R_2: 13.95%, R_1r: 33.35%, R_2r: 13.07%, R_or: 1.25%, R_1R_Z: 0.06%, R_2R_Z: 0.06%, r′r: 0.55%, r″r: 0.53%, and rr: 16.66%.

Estimated Rh phenotype haplotype frequency: R_1: 41.30%, R_2: 16.43%, R_O: 0.63%, R_Z: 0.06%, r′: 0.28%, r″: 0.26%, r: 41.05%.

The JK phenotype distribution of modern Ireland (n = 3423) was: Jk(a+b+): 49.63%, Jk(a−b+): 23.34%, Jk(a+b−): 27.02%.

3.2 | Irish born donors (n = 2939)

The ABO phenotype distribution for BiI donors was: Group A: 28.92%, Group B: 11.13%, Group O: 57.43% and Group A,B: 2.52%. RhD positive: 82.34% and RhD negative: 17.66%. Refer to Table 1 for the totals for the eight ABO groups for the BiI donors for each county and province. The Rh phenotype for the BiI donors can be found in Table 2 with county/provincial totals.

The estimated Rh phenotype haplotype was: (n = 2939): R_1: 40.68%, R_2: 16.81%, R_O: 0.61%, R_Z: 0.05%, r′: 0.19%, r″: 0.31%, r: 41.36%.

The JK phenotype was: Jk(a + b+): 49.64%, Jk(a-b+): 23.10% and Jk(a + b-): 27.26%. Refer to Table 3 for the BiI JK distribution for counties / provinces.

The BiI donors comprised 85.76% of the first time donors. These donors came from Leinster (48.38%), Munster (33.65%), Connacht (11.91%), and Ulster (6.06%).

The percentage of BiI donors recorded from each province, broadly reflected Ireland's population distribution where Leinster comprised 55.3% of the population, followed by Munster 26.9%, Connacht 11.6%, and Ulster at 6.2%.[35]

3.3 | Non Irish born donors (n = 488)

The ABO phenotype distribution for the BoI donors was: Group A: 35.25%, Group B: 17.42%, Group O: 39.96% and Group A,B: 7.38%. RhD positive: 81.76% and RhD negative: 18.24%. Refer to Table 4 for the totals for the eight ABO blood group frequencies and Table 5 for the Rh phenotype of the BoI donors by geographical region.[23]

Estimated Rh phenotype haplotype (n = 488): R_1: 45.09%, R_2: 14.14%, R_O: 0.72%, R_Z: 0.10%, r′: 0.82%, r″: 0.00%, r: 39.15%.

The JK phenotype was: Jk(a+b+): 49.59%, Jk(a−b+): 24.80% and Jk(a+b−): 25.61%. The JK BGS distribution by each geographical region can be found in Table 6.

The four largest groups of BoI donors were found in Europe split into four regions[23]: Northern: 27.5%, Eastern: 26.43%, Southern: 11.48%, and Western: 12.30%. The other 110 donors came from various parts of the World. Asian donors accounted for 9.22%, Oceania 3.07%, Africa 1.64%, and North American donors comprised 8.61% of the BoI donors.

3.4 | BoI donor participation in the study relative to their proportion in the population

Refer to Table 7 for the estimated proportions in the Irish population of each geographical area where the BoI donors from this

TABLE 1 ABO BGS distribution for BIl donors

	A+	A-	B+	B-	O+	O-	AB+	AB-	Total
Leinster									
Carlow	8	1	5	1	14	1	0	0	30
Dublin city	98	22	44	12	210	41	6	3	436
Dublin	45	15	17	4	108	23	4	0	216
Kildare	23	7	16	3	52	16	2	0	119
Kilkenny	24	7	8	3	33	7	3	1	86
Laois	11	1	3	0	27	3	3	0	48
Longford	0	3	0	0	6	0	0	0	9
Louth	15	5	5	1	32	6	0	0	64
Meath	25	6	11	4	45	9	2	0	102
Offaly	10	1	6	5	26	5	1	0	54
Westmeath	14	2	4	2	27	3	4	0	56
Wexford	36	3	5	3	55	17	4	2	125
Wicklow	22	8	4	1	32	8	1	1	77
Sub-Total	331	81	128	39	667	139	30	7	1422
%	23.28	5.70	9.00	2.74	46.91	9.77	2.11	0.49	100
Munster									
Clare	20	1	5	0	32	12	1	0	71
Cork	109	24	37	9	214	52	7	0	452
Kerry	18	5	13	1	59	7	2	1	106
Limerick	29	5	8	2	53	7	5	0	109
Tipperary	41	6	12	0	55	13	4	1	132
Waterford	31	5	13	4	50	14	1	1	119
Sub-Total	248	46	88	16	463	105	20	3	989
%	25.08	4.65	8.90	1.62	46.81	10.62	2.02	0.30	100
Connacht									
Galway	29	5	15	2	82	14	3	2	152
Leitrim	3	1	3	0	14	3	0	0	24
Mayo	22	5	6	0	46	9	1	0	89
Roscommon	11	1	3	1	19	4	0	0	39
Sligo	5	0	2	1	28	7	1	2	46
Sub-Total	70	12	29	4	189	37	5	4	350
%	20.00	3.43	8.29	1.14	54.00	10.57	1.43	1.14	100
Ulster (part of)									
Cavan	16	5	4	0	22	3	1	1	52
Donegal	25	2	16	1	33	6	3	0	86
Monaghan	10	4	2	0	20	4	0	0	40
Sub Total	51	11	22	1	75	13	4	1	178
%	28.65	6.18	12.36	0.56	42.13	7.30	2.25	0.56	100
Total	700	150	267	60	1394	294	59	15	2939
%	23.82	5.10	9.08	2.04	47.43	10.00	2.01	0.51	100

study indicated origin.[36] The populations of these geographical regions where the BoI donors indicated origin comprised an estimated 10% of Ireland's total resident population, with the European regions comprising almost 9% of this figure. However, with a sample size of 488 BoI donors, less than 0.10% of the BoI donor's resident in Ireland participated.

3.5 | Analysis

The data was assessed using an urban/rural analysis format to determine if there were differences of the relevant distributions within Ireland. Further analysis established if there were

TABLE 2 Rh phenotype distribution for BiI donors

	R1R1	R1R2	R2R2	Rl1r	R2r	Ror	r′r	r″r	rr	Other	Total
Leinster											
Carlow	7	6	1	10	3	0	0	0	3	0	30
Dublin city	73	56	15	144	63	7	1	5	74	0	436
Dublin	41	27	5	79	21	1	0	1	41	0	216
Kildare	19	14	7	36	17	0	0	0	26	0	113
Kilkenny	11	8	4	39	6	0	0	1	17	0	86
Laois	14	4	0	17	9	0	0	0	4	0	48
Longford	2	0	0	2	1	1	0	0	3	0	9
Louth	8	14	1	22	7	0	0	1	11	0	64
Meath	16	17	1	30	17	2	1	0	18	0	102
Offaly	5	6	0	20	12	0	0	0	11	0	54
Westmeath	12	9	1	20	5	2	0	0	7	0	56
Wexford	15	19	4	40	21	1	0	0	25	0	125
Wicklow	14	7	3	24	8	3	0	0	18	0	77
Sub-Total	237	187	42	483	190	17	2	6	258	0	1422
%	16.67	13.15	2.95	33.97	13.36	1.20	0.14	0.42	18.14	0.00	100
Munster											
Clare	10	15	3	17	12	1	0	0	13	0	71
Cork	78	61	13	150	59	4	6	4	75	2[a]	452
Kerry	20	12	1	38	19	2	1	2	11	0	106
Limerick	22	12	4	37	16	3	0	1	13	1[b]	109
Tipperary	15	27	7	42	18	3	0	2	18	0	132
Waterford	12	19	5	42	16	1	2	0	22	0	119
Sub-Total	157	146	33	326	140	14	9	9	152	3	989
%	15.87	14.76	3.34	32.96	14.16	1.42	0.91	0.91	15.37	0.30	100
Connacht											
Galway	26	22	4	55	20	2	0	2	21	0	152
Leitrim	4	5	0	8	2	1	0	0:	4	0	24
Mayo	13	14	4	32	12	0	0	0	14	0	89
Roscommon	5	13	1	9	5	0	0	0	6	0	39
Sligo	6	7	2	15	6	0	0	0	10	0	46
Sub-Total	54	61	11	119	45	3	0	2	55	0	350
%	15.43	17.43	3.14	34.00	12.86	0.86	0.00	0.57	15.71	0.00	100
Ulster (part of)											
Cavan	11	5	0	21	5	1	0	0	9	0	52
Donegal	21	11	0	35	9	1	0	0	39	0	86
Monaghan	9	8	2	9	4	0	0	1	7	0	40
Sub Total	41	24	2	65	18	2	0	1	25	0	178
%	23.03	13.48	1.12	36.52	10.11	1.12	0.00	0.56	14.04	0.00	100
Total	489	418	88	993	393	36	11	18	490	3	2939
%	16.64	14.22	2.99	33.79	13.37	1.22	0.37	0.61	16.67	0.10	100

[a] R_1R_z by one and R_2R_z by one.
[b] R_1R_z by one.

differences in the resultant two populations. Analysis of the observed vs calculated expected values from the allele frequencies was performed. An analysis with that expected from previous studies[4,6,15-17] to the observed values obtained in this 2015 snapshot was undertaken for ABO and RhD distributions.

TABLE 3 Kidd BGS distribution for BiI donors

	Jk(a+b+)	Jk(a-b+)	Jk(a+b-)	Total
Leinster				
Carlow	17	7	6	30
Dublin city	220	95	118	433[a]
Dublin	108	61	46	215[b]
Kildare	66	22	31	119
Kilkenny	35	26	25	86
Laois	25	6	17	48
Longford	3	4	2	9
Louth	40	10	14	64
Meath	54	22	26	102
Offaly	25	15	14	54
Westmeath	26	16	14	56
Wexford	57	28	40	125
Wicklow	40	21	16	77
Sub-Total	716	333	369	1418
%	50.49	23.48	26.02	100
Munster				
Clare	32	17	22	71
Cork	237	97	118	452
Kerry	41	29	36	106
Limerick	53	25	31	109
Tipperary	65	31	36	132
Waterford	52	25	42	119
Sub-Total	480	224	285	989
%	48.53	22.65	28.82	100
Connacht				
Galway	65	42	45	152
Leitrim	17	5	2	24
Mayo	49	21	19	89
Roscommon	18	8	13	39
Sligo	29	8	9	46
Sub-Total	178	84	88	350
%	50.86	24.00	25.14	100
Ulster (part of)				
Cavan	24	10	18	52
Donegal	38	20	28	86
Monaghan	21	7	12	40
Sub Total	83	37	58	178
%	46.63	20.79	32.58	100
Total	1457	678	800	2935
%	49.64	23.10	27.26	100

[a]No data by three.
[b]No data by one.

3.5.1 | Irish born donors data analysis

The Irish ABO distribution was analyzed using chi square for urban / rural association. Using a 7 × 8 contingency table (Supplementary Table: S1) for Dublin, Leinster, Cork, Munster, Galway, Connacht, and Ulster and the eight blood group totals, no statistically significant differences were found between the ABO distribution and geographical areas within Ireland. $\chi 2 = 40.58$, 42df, *P*-value = 0.534.

The same regions (Dublin, Leinster, Cork, Munster, Galway, Connacht, and Ulster) were analyzed for RhD positive vs RhD negative association using a 7 × 2 contingency table (S2). No statistically significant association was found between the urban and rural areas with regards to D antigen distribution. $\chi 2 = 4.63$, 6df, *P*-value = 0.592.

A rural/urban analysis using a 7 × 3 (Dublin, Leinster, Cork, Munster, Galway, Connacht, and Ulster) contingency table (S3) was used to examine the JK distribution within Ireland. No statistically significant difference was found in this distribution within the geographical areas of Ireland. $\chi 2 = 18.45$, 12df, *P*-value = 0.103.

Another rural/urban analysis was used to examine the Rh phenotype distribution throughout Ireland using a 7 × 10 (Dublin, Leinster, Cork, Munster, Galway, Connacht, and Ulster) contingency table. (S4). No evidence of any statistically significant differences in Rh phenotype distribution within Ireland was found. $\chi 2 = 57.78$, 54df, *P*-value = 0.338.

3.5.2 | Irish born vs non-Irish born donor data analysis

The BiI vs BoI donor population was analyzed for the eight ABO blood groups, using a 2 × 8 contingency table (S5). On analysis, a highly statistically significant difference: ($\chi 2 = 78.42$, 7df, *P*-value <0.001), was found which indicated a strong difference between the two populations in regard to the ABO blood group distribution, the result being a relative decrease in Group O and a relative increase in Group A, B and Group A,B in the BoI donor population.

Both populations were analyzed for RhD positive vs RhD negative associations, using a 2 × 2 contingency table (S6). No statistically significant difference was found. $\chi 2 = 0.10$, 1df, *P*-value = 0.757.

The Rh phenotype distribution between the two populations was analysed in a 2 × 10 contingency table (S7); a highly statistically significant difference ($\chi 2 = 31.48$, 9df, *P*-value < 0.001) was noted for the populations in regard to RHCE distributions; the two largest single degree of freedom components were: R_1R_1 for BiI donors (16.64%) vs BoI donors (23.57%) where $\chi 2 = 13.83$, 1df, *P*-value < 0.001 and for r′r for BiI donors (0.37%) vs BoI donors (1.64%) where $\chi 2 = 12.08$, 1df, *P*-value = 0.001.

The JK distribution of both populations was analyzed using a 2 × 3 contingency table (S8); $\chi 2 = 0.93$, 2df, *P*-value = 0.627; therefore, no evidence of a statistically significant difference between the two populations was found.

3.5.3 | Expected Allele frequency calculations (HWE) and analysis

For the allele frequencies for the relevant BGS's refer to Table 8. The observed and estimated expected allele values[29-34] are found in Table 9.

TABLE 4 ABO BGS distribution for BoI donors

	A+	A-	B+	B-	O+	O-	AB+	AB-	Total
Northern Europe									
Total	36	6	15	4	56	12	2	2	133
%	27.07	4.51	11.28	3.01	42.11	9.02	1.50	1.50	100
Eastern Europe									
Total	33	19	18	8	28	5	15	3	129
%	25.58	14.73	13.95	6.20	21.71	3.88	11.63	2.33	100
Southern Europe									
Total	14	5	5	2	24	2	4	0	56
%	25.00	8.93	8.93	3.57	42.86	3.57	7.14	0.00	100
Western Europe									
Total	21	7	8	0	16	4	4	0	60
Total %	35.00	11.67	13.33	0.00	26.67	6.67	6.67	0.00	100
Eastern Asia	2	0	3	0	3	0	1	0	9
Central Asia	1	0	0	0	0	0	0	0	1
Southern Asia	1	0	0	0	1	0	0	0	2
South East Asia	3	0	3	0	7	0	1	0	14
Western Asia	4	0	7	0	6	1	1	0	19
Northern America	11	2	6	2	16	3	2	0	42
Oceania	5	0	2	0	6	1	1	0	15
Africa	2	0	1	1	4	0	0	0	8
Overall Total	133	39	68	17	167	28	31	5	488
Overall %	27.25	7.99	13.93	3.48	34.22	5.74	6.35	1.02	100

Chi square analysis for the ABO BGS frequency for the observed vs expected values[29] were consistent with the population been in Hardy Weinberg equilibrium for the total, BiI and BoI populations as the *P*-value was greater than *.10* for each analysis,[29] which indicated no statistically significant difference between the observed and the expected ABO groups calculated for each population.

The RhD phenotype was determined serologically as RhD positive and RhD negative, however, the expected values for RhD+ hemizygous vs homozygous expression were calculated from the observed allele frequencies (Table 9) for each population.[30,31] Expected JK allele estimates were also calculated from the observed allele frequencies for each population. [32-34] Chi square analyses between the observed and expected D values and JK values (Table 9) indicated agreement for each donor population.

3.5.4 | Expected BGS prevalence vs observed BGS prevalence

The observed prevalence (total and BiI) with that expected can be found in Table 10. The differences considered clinically relevant for this study were 5.0% for all groups except, 3.0% for Group B and 1.0% for Group A,B. All observed values were within 95% confidence limits of the expected values, calculated at 80% and 90% power.[24-26] 80% power was achieved for Group O and Jk(a+b+) phenotypes and 90% power was achieved for RhD antigen sample size for this study. The difference between the observed and expected (Table 10) was not considered clinically relevant.

3.5.5 | Analysis of observed values with previous studies

Previous study frequencies, plus the author's data for total (n = 3427), BiI (n = 2939), and BoI (n = 488) are in Table 11. It can be observed that there is variability in regard to ABO frequencies between the previous studies and between the current study and the previous studies.

Contingency tables for two previous studies[6,15] were prepared; these were the only two studies with total numbers that could be tabulated for the eight ABO groups in the same format as current study. On analysis in a 3 × 8 contingency table (S9), statistical significant differences were found: $\chi 2 = 30.69$, 14 df, *P*-value = 0.006. However, when the current study was compared to the 1956 study[6] and the 1964 study[15] in 2 × 8 contingency tables (S10 and S11), statistical significance was found, for the 1956 study[6]; ($\chi 2 = 15.84$, 7df, *P*-value = 0.027) but not for the 1964 study[15] ($\chi 2 = 6.27$, 7df, *P*-value = 0.517).

An earlier (1940)[4] and later (1977)[17] study was analyzed, with the current study, using 2 × 4 contingency tables (S12 and S13). A

TABLE 5 Rh phenotype BGS distribution for BoI donors

	R1R1	R1R2	R2R2	R1r	R2r	Ror	r′r	r″r	rr	Other	Total
Northern Europe											
Total	25	23	5	36	18	2	4	0	20	0	133
%	18.80	17.29	3.76	27.07	13.53	1.50	3.01	0.00	15.04	0.00	100
Eastern Europe											
Total	32	8	3	39	11	0	4	0	31	1[a]	129
%	24.81	6.20	2.33	30.23	8.53	0.00	3.10	0.00	24.03	0.78	100
Southern Europe											
Total	15	3	0	25	4	0	0	0	9	0	56
%	26.79	5.36	0.00	44.64	7.14	0.00	0.00	0.00	16.07	0.00	100
Western Europe											
Total	15	5	0	20	8	1	0	0	11	0	60
Total %	25.00	8.33	0.00	33.33	13.33	1.67	0.00	0.00	18.33	0.00	100
Eastern Asia	4	2	1	0	2	0	0	0	0	0	9
Central Asia	0	0	0	1	0	0	0	0	0	0	1
Southern Asia	0	1	0	1	0	0	0	0	0	0	2
South East Asia	9	4	0	1	0	0	0	0	0	0	14
Western Asia	5	3	0	5	2	3	0	0	1	0	19
Northern America	8	7	2	12	6	0	0	0	7	0	42
Oceania	0	2	0	8	4	0	0	0	1	0	15
Africa	2	2	0	2	0	1	0	0	1	0	8
Overall Total	115	60	11	150	55	7	8	0	81	1	488
Overall %	23.57	12.30	2.25	30.74	11.27	1.43	1.64	0.00	16.60	0.20	100

[a]R_2R_z by one.

statistically significant difference was found for the 1940[4] study; ($\chi 2 = 8.76$, 3df, *P*-value = 0.033), but not for the 1977[17] study; ($\chi 2 = 0.53$, 3df, *P*-value = 0.912).

An assessment of RhD frequency change over time was performed using two contingency tables in 2 × 2 formats (S14 and S15) for the 1948[16] study; ($\chi 2 = 2.45$, 2df, *P*-value = 0.117) and 1964 study[15] ($\chi 2 = 1.15$, 2df, *P*-value = 0.283). These were the only two studies with total numbers for tabulations. No statistical significance was found for the RhD antigen distribution from analysis of these previous studies.

4 | DISCUSSION

The observed phenotype frequencies have remained relatively unchanged from the prevalence values expected[13-16] for the study (Table 10), however, variability was observed between the previous studies[3-6,14-17] and between this study and the past studies (Table 11). From the available evidence, statistical significance was found for the ABO distribution between this study and some of the previous studies but not for the RhD antigen distribution.

The BoI donor population was statistically significantly different to the BiI population in relation to aspects of the ABO and Rh phenotype distribution. This has service implications for the IBTS; in terms of patient requirements, as there is a cohort of sickle cell anemia patients of African ancestry on transfusion programs for primary prophylaxis of cerebrovascular disease where Group B frequency at greater than 20% are found.[13] Group O is often substituted for Group B for this cohort of patients leading to pressures on the Universal group.

Low numbers of donors came from the African continent (1.64%). Increased recruitment of these donors would broaden the choice of blood groups available to ensure matching of groups to patients and might help to avoid overuse of O negative units (rr substituted for R_or or R_0R_0). O negatives accounted for 9.40% of the Irish blood supply according to this study. This is of concern and the IBTS has a business objective that the BAME community (Black, Asian, and Minority Ethnic populations) will be targeted for blood donation, post introduction of malaria testing at the IBTS.

Most haemoglobinopathy and thalassemia patients require extended phenotyping due to regular transfusions and exchanges.[21] Many of these patients require four to eight units twice a month, with an exchange transfusion requiring 10 units. With increased recruitment of African born donors an increase in R_or blood products would be expected. R_or prevalence for these donors is 45.8% whereas for Caucasians the R_or prevalence is 2.1%.[13] From this study, R_or phenotypes comprised 1.22% of BiI donors and 1.43% of BoI donors. The provision of R_or blood cell products presents a significant challenge

for blood stock management. The frequency of 45% R_or prevalence in populations of African ethnicity does not impact on the Irish supply because of low participation of this group in blood donation. The 2016 census reported 64 639 citizens of African ethnicity resident in Ireland.[37]

The ABO BGS of the BoI donors had increased Group A, Group B and Group A,B and less Group O than the BiI donors. This fact was particularly noticeable for the Eastern European donors who comprised 26.43% of the BoI donors (Table 4). A limitation of this data was n = 129, however, increased recruitment of these donors might assist in the provision of ABO matched blood groups. Poland, a country in the eastern European geographical area, accounted for the largest non-Irish national group in Ireland with a population of 122 515.[8]

The first time donor's BoI comprised 14.24% of the donor population in this study, which is positive for the IBTS in terms of donor geographical diversity to enable provision of units for those patients with complex transfusion requirements. However, it was demonstrated that the European cohort (378 donors: 77.46%) had compensated for the Non-European cohort (110 donors: 22.54%). This was reflective of the non-Irish population living in Ireland corresponding to the geographical location of the BoI donor population (Table 7).

Based on the number of BoI donors who donated during this study, an estimated 1800 BoI donors could be expected to donate to the IBTS in a year; this might be increased substantially with a proposed targeted advertising campaign aimed to recruit more donors from all World regions, in particular, to encourage the non-European cohort.

The Jk(a-b-) phenotype can be found at highest frequencies in Polynesian donors where frequency occurs at 0.9%.[13] With few donors from this World region, this rare Jk(a−b−) phenotype was not observed in this 2015 snapshot of 3423 donors. No evidence of Jk(a$+^w$b−) phenotype was observed. A small number of donors (0.38%) were at the threshold of detection with a weakened Jk^b antigen expression, all had heterozygous antigen expression.

TABLE 6 Kidd BGS distribution for BoI donors

	Jk(a+b+)	Jk(a-b+)	Jk(a+b-)	Total
Northern Europe				
Total	69	34	30	133
%	51.88	25.56	22.56	100
Eastern Europe				
Total	65	33	31	129
%	50.39	25.58	24.03	100
Southern Europe				
Total	27	13	16	56
%	48.21	23.21	28.57	100
Western Europe				
Total	31	12	17	60
Total %	51.67	20.00	28.33	100
Eastern Asia	4	1	4	9
Central Asia	1	0	0	1
Southern Asia	2	0	0	2
South Eastern Asia	3	5	6	14
Western Asia	9	5	5	19
Northern America	20	10	12	42
Oceania	7	5	3	15
Africa	4	3	1	8
Overall Total	242	121	125	488
Overall %	49.59	24.80	25.61	100

TABLE 7 BoI donor participation relative to their proportion in the Irish population

Geographical origin of the BoI donors[23]	Non-Irish Donors	% BoI population resident in Ireland from each Geographical Origin[36]		% resident in Ireland from each geographical area as % of the total Irish population		% participation of BoI donors in study as % of each geographical area resident in Ireland	
	N	2011[a]	2016[b]	2011[a]	2016[b]	2011	2016
N Europe	133	174 676	164 295	3.86	3.50	0.08	0.08
E Europe	129	173 173	182 754	3.83	3.90	0.07	0.07
S Europe	56	19 160	35 461	0.42	0.76	0.29	0.16
W Europe	60	27 823	30 680	0.61	0.65	0.22	0.20
E Asia	9	12 616	11 702	0.28	0.25	0.07	0.08
C Asia	1	125	125	0.00	0.00	0.80	0.80
S Asia	2	3218	1958	0.07	0.04	0.44	0.72
SE Asia	14	7213	16 086	0.16	0.34	0.03	0.01
W Asia	19	2918	4545	0.06	0.10	0.65	0.42
N America	42	13 404	12 985	0.30	0.28	0.31	0.32
Oceania	15	4243	3524	0.09	0.08	0.35	0.43
Africa	8	9342	6442	0.21	0.14	0.09	0.12
Total	488	447 911	470 557	9.90	10.03	0.11	0.10

[a]Population usually resident in Ireland 2011: 4 525 281.[36]
[b]Population usually resident in Ireland 2016: 4 689 921.[36]

TABLE 8 Allele frequency values

	A[29]	B[29]	O[29]	RhD+[30,31]	RhD-[30,31]	JK*A[32-34]	JK*B[32-34]
All Ireland	0.1796	0.0772	0.7432	0.5788	0.4212	0.5184	0.4816
BiI	0.1715	0.0702	0.7584	0.5798	0.4202	0.5208	0.4792
BoI	0.2360	0.1258	0.6382	0.5729	0.4271	0.5041	0.4959

TABLE 9 Observed vs calculated expected allele values

	Total (n=3427)		BoI (n=488)		BiI (n=2939)	
Allele	**Observed**	**Expected[29]**	**Observed**	**Expected[29]**	**Observed**	**Expected[29]**
A	1022	1022	172	172	850	850
B	412	424	85	91	327	330
o	1883	1883	195	195	1688	1688
A,B	110	98	36	30	74	71
		Expected[30,31]		Expected[30,31]		Expected[30,31]
RhD+ (homozygous)[a]	1185	1148	187	160	998	988
RhD+ (hemizygous)[a]	1634	1671	212	239	1422	1432
RhD-	608	608	89	89	519	519
		Expected[32-34]		Expected[32-34]		Expected[32-34]
*JK*A*	925	920	125	124	800	796
*JK*B*	799	794	121	120	678	674
*JK*AJK*B*	1699	1709	242	244	1457	1465

[a]Predicted phenotype.

TABLE 10 Expected prevalence vs observed prevalence

Blood group phenotypes	Expected prevalence %	Observed % Total n=3427	Observed% BiI n=2939
O	56[14,15]	55	57
A	31[14,15]	30	29
B	11[14,15]	12	11
AB	3[14,15]	3	3
D	83[14-16]	82	82
Jk(a-b+)	23[13]	23	23
Jk(a+b-)	26[13]	27	27
Jk(a+b+)	50[13]	50	50

Previous Irish studies and this current study agree that Group O had a higher frequency in the west of Ireland (greater than 60% in Connacht). For example, clinics in the west of Ireland would yield more Group O donors. A higher percentage of Group A in Ulster, relative to the other provinces (Table 1) agreed with previous observations.[6] Previous studies noted higher rates of Group B, in particular in relation to Roscommon and Longford.[38] There was insufficient data from these counties to assess this, however, the Ulster province (n = 178) recorded highest Group B (12.92%).

Rh phenotype data from a previous Irish study[17] agreed with this 2015 study (Table 2) which showed higher R_1R_2 complexes in Connacht (17.43%) and also indicated a higher prevalence of rr donors in Leinster (18.14%).

Individual data from counties in Ireland for the BiI donors was insufficient to analyze differences between the counties and provinces in more detail so one would need a larger study with more data from all counties to observe these trends. A larger study on the BoI donors would give stronger evidence of an overall difference of this population to the Irish population, with larger sample sizes from all the geographical areas.

A significant limitation of this study was that the ethnic background of the donor could not be captured on the Health and Lifestyle questionnaire (HLQ), therefore BiI donors who were not of white Irish ethnicity were not identified; the same applied to BoI donors who may in fact have been of Irish ethnicity but BoI. Many people may be born in for example, Africa, but state that their nationality is Irish and vice versa. Dual nationalities have increased from 55 905 in 2011 to 104 784 in 2016.[39] However, in the absence of ethnicity data the BoI measure is used as the best surrogate available. The IBTS have plans to capture donor ethnicity on the HLQ's to enable selection of donors for extended phenotyping and to identify rare donors for specific screening.

5 | CONCLUSION

3427 donors with a full Rh phenotype and 3423 donors with a Kidd type were added to the IBTS donor database; these donors were available for further extended phenotyping on re-donation. The observed phenotype frequencies for the relevant BGS's remained relatively unchanged from the prevalence values expected for the study, however, statistical significance was found between this study and

TABLE 11 Previous Irish studies and 2015 study

Study	n	A	B	O	A,B	RhD+	Jk(a+b+)	Jk(a-b+)	Jk(a+b-)
1937[3]	399	31.1	12	55.2	1.7	n/a	n/a	n/a	n/a
1940[4]	2435	32.36	11.46	53.63	2.54	n/a	n/a	n/a	n/a
1947[5]	26 423	33.5	10.8	53.0	2.7	n/a	n/a	n/a	n/a
1948[16]	4058	n/a	n/a	n/a	n/a	83.76	n/a	n/a	n/a
1956[6]	21 894	31.69	11.11	54.49	2.70	83.87	n/a	n/a	n/a
1958[14]	55 696	30.79	10.78	55.75	2.67	83.34	n/a	n/a	n/a
1964[15]	117 287	30.61	10.89	55.89	2.60	83.09	n/a	n/a	n/a
1977[17]	1699	29.13	10.77	57.23	2.82	79.43	n/a	n/a	n/a
Caucasian pop.[13]	n/a	43	9	44	3	85	50.3	23.4	26.3
Authors study	3427	29.82	12.02	54.95	3.21	82.26	49.63	23.34	27.02
Authors study BiI	2939	28.92	11.13	57.43	2.52	82.34	49.64	23.10	27.26
Authors study BoI	488	35.25	17.42	39.96	7.38	81.76	49.59	24.80	25.61

Note: All contingency tables for chi square assessment for this manuscript are found in a separate Supplementary Tables file.

some of the previous studies for ABO distribution. The ABO and Rh phenotype distribution between the BiI and BoI donors was found to be statistically significantly different in aspects of their frequencies; it is these BoI donors that the IBTS hopes to encourage to donate with various campaigns. The outcome is a snapshot of the ABO (001), RH (004), and JK (009) BGSs in modern Ireland.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

COMPETING INTERESTS

The authors have no competing interests.

AUTHOR CONTRIBUTIONS

Conceptualization: Anne Browne,
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All authors have read and approved the final version of the manuscript.

Anne Browne had full access to all data in this study and takes complete responsibility for the integrity of the data and accuracy of the data analysis.

TRANSPARENCY STATEMENT

I Anne Browne affirm that this manuscript is an honest, accurate and transparent account of the study been reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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

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