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# Improving HLA typing imputation accuracy and eplet identification with local next-generation sequencing training data

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Assessing donor/recipient HLA compatibility at the eplet level requires second field DNA typings but these are not always available. These can be estimated from lower-resolution data either manually or with computational tools currently relying, at best, on data containing typing ambiguities. We gathered NGS typing data from 61,393 individuals in 17 French laboratories, for loci A, B, and C (100% of typings), DRB1 and DQB1 (95.5%), DQA1 (39.6%), DRB3/4/5, DPB1, and DPA1 (10.5%). We developed HaploSFHI, a modified iterative maximum likelihood algorithm, to impute second field HLA typings from low- or intermediate-resolution ones. Compared with the reference tools HaploStats, HLA-EMMA, and HLA-Upgrade, HaploSFHI provided more accurate predictions across all loci on two French test sets and four

**Abbreviations:** CI, confidence interval; DNA, deoxyribonucleic acid; FN, false negatives; FP, false positives; HSCT, hematopoietic stem cell transplantation; LD, linkage disequilibrium; NGS, next-generation sequencing.

PHRC Acorghla Consortium: The PHRC HLA Consortium participants to the HaploSFHI project were the HLA laboratories from Amiens (Dr Nicolas Guillaume, Amiens University Hospital, [guillaume.nicolas@chu-amiens.fr](mailto:guillaume.nicolas@chu-amiens.fr)), Angers (Dr Florent Delbos, Etablissement Français du Sang Centre Pays de la Loire, [florent.delbos@efs.sante.fr](mailto:florent.delbos@efs.sante.fr)), Besançon (Dr Anne Dormoy, Etablissement Français du Sang Bourgogne Franche-Comté, [anne.dormoy@efs.sante.fr](mailto:anne.dormoy@efs.sante.fr)), Bordeaux (Dr Line-Gwenda Guidicelli, Bordeaux University Hospital, [line-gwenda.guidicelli@chu-bordeaux.fr](mailto:line-gwenda.guidicelli@chu-bordeaux.fr)), Créteil (Dr Laure Croisille, Etablissement Français du Sang Ile de France, [laure.croisille@efs.sante.fr](mailto:laure.croisille@efs.sante.fr)), Grenoble (Dr Béatrice Bardy, Etablissement Français du Sang Rhône-Alpes [beatrice.bardy@efs.sante.fr](mailto:beatrice.bardy@efs.sante.fr)), La Réunion (Dr Julien Eperonnier, La Réunion University Hospital, [julien.eperonnier@chu-reunion.fr](mailto:julien.eperonnier@chu-reunion.fr)), Lille (Dr Isabelle Top-Raymond, Lille University Hospital, [isabelle.raymond@chu-lille.fr](mailto:isabelle.raymond@chu-lille.fr)) Limoges (Dr Matthieu Filloux, Limoges University Hospital, [matthieu.filloux@chu-limoges.fr](mailto:matthieu.filloux@chu-limoges.fr)), Lyon (Dr Valérie Dubois, Etablissement Français du Sang Rhône-Alpes, [valerie.dubois@efs.sante.fr](mailto:valerie.dubois@efs.sante.fr)), Montpellier (Dr Céline Thévenin, Montpellier University Hospital, [c-rene@chu-montpellier.fr](mailto:c-rene@chu-montpellier.fr)), Nantes (Dr Alexandre Walencik, Etablissement Français du Sang Centre Pays de la Loire, [alexandre.walencik@efs.sante.fr](mailto:alexandre.walencik@efs.sante.fr)), Paris Saint-Louis (CU, DJC, JLT, JS, RL), Poitiers (Dr Isabelle Jollet, Etablissement Français du Sang Nouvelle Aquitaine, [isabelle.jollet@efs.sante.fr](mailto:isabelle.jollet@efs.sante.fr)), Rennes (Dr Virginie Renac, Etablissement Français du Sang Bretagne, [virginie.renac@efs.sante.fr](mailto:virginie.renac@efs.sante.fr)), Saint-Etienne (Dr Valérie Dubois, Etablissement Français du Sang Rhône-Alpes, [valerie.dubois@efs.sante.fr](mailto:valerie.dubois@efs.sante.fr)), and Tours (Dr Barbara Proust, Etablissement Français du Sang Centre Pays de la Loire, [barbara.proust@efs.sante.fr](mailto:barbara.proust@efs.sante.fr)).

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European-independent test sets. Only HaploSFHI could impute DQA1, and solely HaploSFHI and HaploStats provided DRB3/4/5 imputations. The improved performance of HaploSFHI was due to our local and nonambiguous data. We provided explanations for the most common imputation errors and pinpointed the variability of a low number of low-resolution haplotypes. We thus provided guidance to select individuals for whom sequencing would optimize incompatibility assessment and cost-effectiveness of HLA typing, considering not only well-imputed second field typing(s) but also well-imputed eplets.

#### KEYWORDS

eplets, histocompatibility, HLA, imputation, next-generation sequencing (NGS)

## 1 | INTRODUCTION

Transplantations have been repeatedly shown to be improved by a minimal number of HLA mismatches between recipient and donor.<sup>1–5</sup> However, the difficulty resides in the extreme polymorphism of the 8–11 HLA genes located on chromosome 6.<sup>6</sup> Currently, >30,000 DNA alleles differing by at least one nucleotide have been identified. They encode around 20,000 proteins with at least one distinct amino acid.

An HLA allele is named with up to four sets of digits separated by colons (e.g., HLA-B\*44:02:01:02). The first field gathers proteins close in sequence, that is, belonging to the same allelic series, roughly corresponding to the historical serologically defined HLA groups. Alleles sharing the first and second fields will encode a unique protein, that is, a unique amino acid sequence. Alleles that do not have identical third and/or fourth fields only differ by synonymous nucleotide substitutions in the coding region and/or sequence polymorphisms in the introns. Hence, only typings up to the second field are considered when measuring compatibility at the amino acid level. The compatibility between a donor and a recipient is determined by the number of antigens or alleles possessed by the donor and absent in the recipient, among class I HLA-A, -C, -B and class II DRB1, DQA1, DQB1, DPA1, DPB1, as well as the DRB3/4/5 genes that are not present in all individuals. The precision of this measure depends on available information and, therefore, on the typing technique used. It is widely acknowledged among HLA laboratory specialists that in situations involving donor–recipient pairs, high-resolution typing should be utilized whenever possible for making clinical decisions.<sup>7</sup>

Resolution of DeoxyriboNucleic Acid (DNA) based HLA typing has hugely improved over the past 30 years but depending on the emergency level of the typing and/or budget constraints, second field resolution is not

always reachable even for loci of strong clinical interest. This is particularly true for deceased donors, as the typing must be performed expeditiously due to the time constraints imposed by the transplantation process. In such instances, only low- or intermediate-resolution typings on a minimal number of genes are available<sup>7</sup> unless the donor is re-typed posttransplantation, which incurs additional time and financial costs. The lack of second field resolution also carries the potential for errors in the pre-transplant compatibility assessment. Not only has matching for broad antigens (e.g., split antigens B51 and B52 belong to the same broad antigen B5) been shown to be insufficient<sup>2</sup> but also split antigens do not carry enough information. Indeed, HLA antibodies specifically recognize a wide range of epitopes present on the surface of HLA proteins, each being roughly centered on the polymorphic position called the eplet. Unfortunately, alleles with the same first field can possess different eplet repertoires—for example, A\*24:03 does not carry the antibody-verified eplet 166DG, whereas A\*24:02 does.<sup>8</sup>

In the absence of complete typings, estimating the most likely second field typing(s) from lower resolution ones is the only available alternative and is a form of imputation. It usually relies on Linkage Disequilibrium (LD), that is, relatively stable combinations of alleles on a given chromosome (haplotypes) that remain unchanged over generations of individuals in a given geographical population or ethnicity.<sup>9</sup> Imputation based on estimating haplotype frequencies over a training set of individuals is considered a reasonable approach. However, Engen et al.<sup>10</sup> found that 65.4% of 303 s field imputed typings were incorrect on at least one of the five loci (HLA-A, -C, -B, DRB1, DQB1) tested when using HaploStats,<sup>11</sup> the most commonly used program for imputation of high-resolution typing.<sup>7</sup> This suggests that imputation may result in assigning inaccurate eplets to a significant number of individuals, particularly if they do not match the reference population. Although the

importance of the inaccuracy introduced by imputation on eplet mismatches inference is debated among experts, there is a consensus on a need for better imputation tools.<sup>7</sup> There is an inherent error rate that exists with any imputation method, but we believe part of the inaccuracy relies upon (i) the use of a large reference population instead of a specific one and (ii) the use of ambiguous typing data. For instance, most of the HaploStats training set used oligonucleotide probe hybridization that provided limited coverage of HLA genes polymorphic regions.<sup>11</sup> As a result, many second field typing(s) were never distinguished from one another. In fact, current tools do not typically return unambiguous second field typing(s), but rather groups of second field typing(s) referred to as “G” groups. For example, DQB1\*02:01:01G refers to DQB1\*02:01 (found in 10.32% of the individuals typed in the HLA laboratories participating in the present work), DQB1\*02:02 (11.28%), DQB1\*02:04 (< 0.01%), and DQB1\*02:06 (< 0.01%).

To improve HLA typing imputation accuracy to the second field resolution level, we gathered nonambiguous typing data exclusively obtained by next-generation sequencing (NGS) from French HLA laboratories. Using these typings, we developed and trained HaploSFHI that imputes second field typing(s) from lower-resolution ones. Besides, there is no extensive benchmark involving up-to-date imputation tools. For example, Engen et al. only compared HaploStats to the HLA Matchmaker Four Digit Allele Converter Program using a small sample of 303 typings.<sup>10</sup> We aim to address this gap by creating a more comprehensive benchmark that incorporates a wider range of tools and employs larger, independent, and unambiguous test data sets. For this purpose, we decided to remeasure accuracy scores for the most popular imputation tools (HaploStats,<sup>11</sup> HLA-Upgrade from the EasyHLA suite,<sup>12</sup> and HLA-EMMA<sup>13</sup>). We decided to exclude the HLA Matchmaker tool from our panel as Engen et al. not only showed that its performance was significantly lower than HaploStats but also could not provide second field typing(s) for 45.2% of the Caucasian subjects.<sup>10</sup>

## 2 | MATERIALS AND METHODS

### 2.1 | NGS typings database

The local NGS typing data were provided anonymized and were collected into a single database. The HLA laboratories of Amiens, Angers, Besançon, Bordeaux, Créteil, Grenoble, La Réunion, Lille, Limoges, Lyon, Montpellier, Nantes, Paris Saint-Louis, Poitiers, Rennes, Saint-Etienne, and Tours included all the validated NGS typings for solid organ transplantations recipients and

donors, hematopoietic stem cell transplantation (HSCT) recipients, and HSCT donors for samples received between the local implementation of the technique and October 15, 2019, or May 2, 2020, for Paris Saint Louis. HSCT donors from abroad were excluded. NGS typing reagents used over the period of data collection were the NGS-Go AmpX kit (GenDX), the NXType kit (One Lambda), and the Holotype kit (Omixon, Budapest, Hungary). The database does not contain any ambiguous data. The term “local” comes with a caveat: we intentionally did *not* exclude non-Caucasian French data to ensure that the developed tool encompasses accuracy not only for individuals of Caucasian descent but also for individuals from diverse backgrounds whose DNA samples are likely to enter an HLA laboratory in France. By aligning our data collection with the actual diversity observed in practice, we aim to create a tool that is fair and equitable in its applicability. The results below, however, show that this caveat is not detrimental to HaploSFHI's accuracy. The project was approved by the local ethical committee.

### 2.2 | Typing imputation strategy

To predict an individual's second field typing(s), we used a modified iterative maximum likelihood algorithm. First, we iteratively estimated first field haplotype frequencies and second field haplotype frequencies for a given first field haplotype with the training set. The rest of the data set (cf. Table 1), that is, the test set, is used to evaluate the accuracy of the algorithm results. Then, when a typing needs imputation, it is separated into two first field haplotype pairs, and then each is imputed into a second field haplotype. Additional supporting information may be found online in the Data S1 section. To mitigate the limitations of estimating haplotype frequencies from a limited amount of data, we applied our haplotype determination algorithms to three-loci blocks where linkage disequilibrium was known to be highest. Specifically, we used (HLA-A, C, B), (HLA-B, DRB1, DQB1), (HLA-DRB1, DQA1, DQB1), and (HLA-DRB3/4/5, DRB1, DQB1) blocks to predict (HLA-A, C, B), (HLA-DRB1, DQB1), (HLA-DQA1), and (HLA-DRB3/4/5), respectively.

### 2.3 | Comparison with other existing tools

We assessed the performance of HaploSFHI by comparing it to three reference tools: HaploStats,<sup>11</sup> HLA-Upgrade from the EasyHLA suite,<sup>12</sup> and HLA-EMMA.<sup>13</sup> These tools use typing databases from European Caucasian (EURCAU,  $n = 1,242,890$ ) individuals and Caucasian (CAU,

TABLE 1 Number of typings in each set of each genes' subgroup.

	HLA-A -C -B	-B -DRB1 -DQB1	-DRB1 -DQA1 -DQB1	-DRB3/4/5 -DRB1 -DQB1
Training	57,484	54,477	21,488	5313
Test	3844	2145	2145	903
Excluded <sup>a</sup>	65	353	20	49
Total	61393	56,975	23,653	6265

<sup>a</sup>Excluded data correspond to data intended for inclusion in the training set post train/test split but that were ultimately excluded from training our algorithm due to insufficient certainty in their haplotype determination.

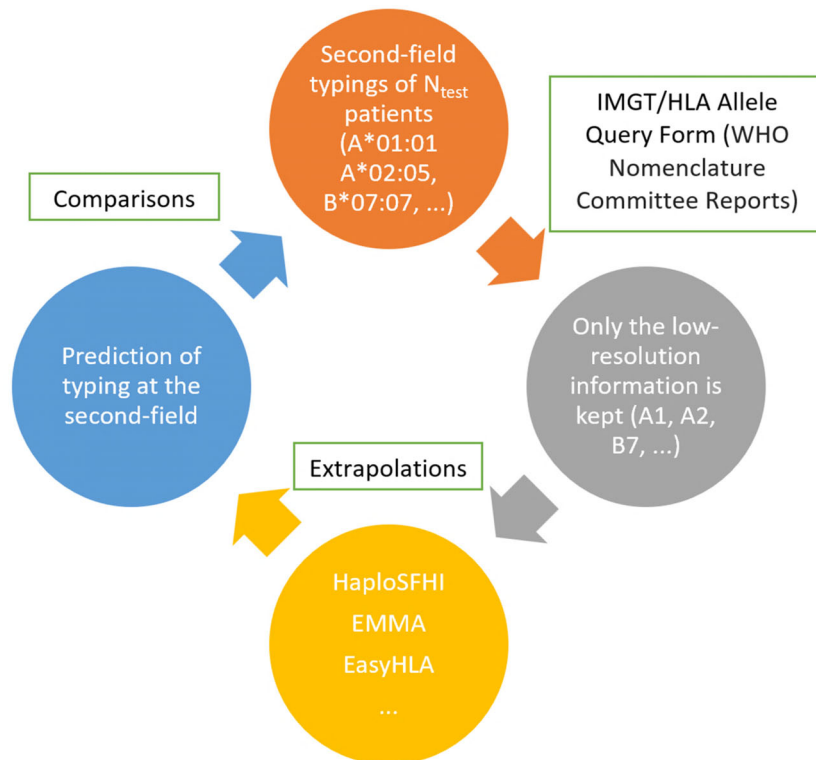


FIGURE 1 Assessing the accuracy of each tool.

$n = 3,912,440$ ) individuals for HaploStats; from European (EU,  $n = 304,697 + 8000$ ) and French (FR,  $n > 8000$ ) individuals for HLA-Upgrade; and from the National Marrow Donor Program ( $n = 81,106$ ) for HLA-EMMA. We implemented an automated process to request imputations on a case-by-case basis for HaploStats since no batch option was available. HLA-EMMA's strategy is slightly different from the two other tools as it does not rely on LD but only returns the most frequent second field typing(s) for a given serological level typing. Only HaploSFHI integrates HLA-DQA1 in its imputation, and only HaploStats and HaploSFHI impute HLA-DRB3/4/5.

To examine performances, second field typing(s) were first converted into serological level typings on five independent test sets: our French test set ( $n = 2135$ , denoted FR-A thereafter), an open-source data set from Nizhny Novgorod,

Russia ( $n = 1510$ ),<sup>14</sup> data set from the Hammer-smith Histocompatibility and Immunogenetics Laboratory, London, United Kingdom (Dr. A. Anand,  $n = 361$ ), a data set from the Scottish National Blood Transfusion Service Histocompatibility and Immunogenetics laboratory, Edinburgh, Scotland (Dr. D. Turner,  $n = 504$ ), and a data set from the Centro De Sangue E Transplantação Do Porto, Porto, Portugal (Dr. S. Tafulo,  $n = 215$ ). We did not convert typings to first field resolution, for example, although B\*15:01 leads to the B15 serological supertype, its serological specificity B62 was retained to better match the current routine reality. Only the second field typing(s) for which no serological specificity has been described to this day were transformed into first field resolution. Then, serological level HLA-A, -C, -B, DRB1, and DQB1 typings were input in the four tools. Second field HLA-A, -C, -B, DRB3/4/5, DRB1,

DQA1, and DQB1 typings were returned, and a first metric, accuracy, was calculated as the percentages of correctly imputed typings. The procedure is summed up in Figure 1.

We also collected typings ( $n = 357$ , denoted FR-B thereafter) from individuals typed at Saint-Louis Hospital, first with NGS for the 11 loci, then confirmed with sequence-specific oligonucleotide (SSO, One Lambda) technology, that is, at an intermediate or NMDP codes resolution level, for A, B, DRB1, and sometimes DQB1. These typings were performed between January 1, 2021 and June 23, 2023, to ensure no overlap with the original training data set of any tools. This allowed us to measure the different tools' accuracies when inferring high-resolution typings from intermediate-resolution ones. To accommodate HaploSFHI, NMDP codes were first translated into lists of ambiguities, as HaploSFHI does not accept NMDP codes directly as input. To our knowledge, HLA EMMA does not impute intermediate-resolution data, so it was excluded from this benchmark. HLA Upgrade FR runs did not go through despite repeated attempts, so it was excluded as well.

HaploSFHI, HaploStats, and HLA-Upgrade provide for each input more than one output, corresponding to the most likely second field typing(s) and possible alternatives that contain distinct second field results for at least one locus. In the present work, we focused on the first-choice output.

We made sure to open source each tool's inputs and outputs for reproducibility purposes (<https://github.com/JasonMendoza2008/HLATypingImputationBenchmarks>) for the Nizhny Novgorod data set, as it could legally be shared.

## 2.4 | Eplet analysis

In addition to this first metric, we quantified the second field typing(s) prediction errors at the eplet level on the FR-A test set. For erroneous second field typings, we analyzed eplets that were not present on the predicted allele but were on the true allele (false negatives [FN]) and eplets that were present on the predicted allele but were not on the true allele (false positives [FP]). We

determined the average number of FN and FP produced by each imputation tool using the open-source Python Package *pelc* v0.2.4.<sup>15</sup> The test set second field typing(s) provided by the four tools and by the NGS techniques were converted into eplets by using the epitope registry,<sup>16</sup> considering all the eplets listed in the registry, version 2022-12-20.

## 2.5 | Confidence intervals

A nonparametric bootstrap method was applied to estimate 95% Confidence Intervals (CIs) for the metrics (accuracies, FN, FP) of each tool. Additional supporting information may be found online in the Data S1 section.

## 3 | RESULTS

### 3.1 | Typing data collection results

We collected NGS typing data from 61,393 individuals in 17 French laboratories. The typings contained, at the second field level, loci A, B, and C (100% of typings), together with DRB1 and DQB1 (95.5%), and with DQA1 (39.6%), DRB3/4/5, DPB1, and DPA1 (10.5%). NGS techniques were implemented for several years, but for a progressively increasing number of loci, resulting in less data for HLA-DRB3/4/5, DQA1, DPA1, and DPB1. Table 1 describes the distribution of the typings according to the three-loci blocks defined in the Materials and methods section. Few typings within the training set, corresponding to "rare" haplotypes, could not be used successfully by our algorithm and were therefore excluded. All evaluations of the algorithm's performance were conducted solely on the testing data set where no exclusions were made. Therefore, this exclusion of rare haplotypes did not introduce any bias in the testing process. Table 2 presents the number of distinct second field typing(s) encountered per locus and compares it to the HaploStats EURCAU database.<sup>11</sup> This latter database consists of

TABLE 2 Number of alleles observed in the HaploSFHI database and in the HaploStats EURCAU database.

	HLA -A	-C	-B	-DRB3/4/5	-DRB1	-DQA1	-DQB1	-DPA1	-DPB1
HaploSFHI									
Observed $\geq 1$ time	226	<b>214</b>	305	31	169	<b>31</b>	<b>80</b>	<b>17</b>	<b>84</b>
Observed $\geq 10$ times	59	44	61	11	105	20	22	10	31
HaploStats EURCAU									
Observed $\geq 1$ time	<b>317</b>	184	<b>530</b>	<b>32</b>	<b>278</b>	0	41	0	0

Note: Bold font depicts the dataset with the most distinct alleles for each locus.

individuals typed at loci HLA-A-B-DRB1, half of which were also typed for HLA-C, nearly half typed for HLA-DRB3/4/5, and almost 13% typed for HLA-DQB1. Interestingly, although systematically including a larger number of individuals typed at any locus and representing a broader population, the HaploStats EURCAU database did not display for each locus the greatest number of distinct alleles. A detailed description of all second field typing(s) identified in our database and their relative frequencies is available in Tables S1–S9.

### 3.2 | Performance of second field typing(s) imputation

HaploSFHI showed the highest performance in predicting second field typing(s) of individuals from French laboratories for all the loci tested on the FR-A data set (Table 3A). HaploSFHI uses the same imputation strategy as HLA-EMMA when encountering an unknown haplotype that cannot be imputed using LD. Therefore, both always output a result. In contrast, HaploStats CAU did not return any result for 0.4% and 1.4% of subjects for loci HLA-A, C, B, DRB1, DQB1, and DRB3/4/5, respectively. For HaploStats EURCAU, they represented 1.7% and 6.4%, respectively. Because of the limited size of HLA-Upgrade FR database, more typings could not be imputed: 9.3% of loci HLA-A, C, B, DRB1, DQB1. This was not the case for HLA-Upgrade EU (0.5%).

HaploSFHI average accuracy on Russian data ( $n = 1510$ , Table 3B), West London data ( $n = 361$ , Table 3C), Scottish data ( $n = 504$ , Table 3D), and Portuguese data ( $n = 215$ , Table 3E) was systematically higher than other tools. This higher performance on those datasets is mainly due to loci HLA-C and -DQB1.

As imputation from intermediate-resolution typings is also relevant, as the resolution level of input data may vary, we analyzed the performance of HaploSFHI and the other available tools on the FR-B data set, an independent French validation data set with SSO resolution typing data as inputs ( $n = 357$ ). Once again, HaploSFHI demonstrated greater efficiency compared with the other tools (Table 3F). Accuracies on locus HLA-C were low for all tools because no information was inputted for locus HLA-C in this benchmark (HLA-C being not typed in the second determination process).

### 3.3 | Distribution of second field typing(s) imputation errors

We investigated which typings were the least correctly imputed on the FR-A test set. Accuracy for each input

TABLE 3A Percentages (and 95% CIs) of typings correctly imputed at the second field resolution level from the HaploSFHI test set ( $n = 3844$  for class I,  $n = 2145$  for class II except HLA-DRB3/4/5 for which  $n = 903$ ).

	HLA-A	-C	-B	-DRB3/4/5	-DRB1	-DQAI	-DQB1	Average
HaploStats CAU	93.05 [92.25–93.85]	93.68 [92.93–94.43]	90.26 [89.38–91.14]	71.69 [69.94–73.44]	78.88 [77.65–80.11]	NA	78.62 [77.38–79.86]	86.90
HaploStats EURCAU	91.75 [90.84–92.66]	92.45 [91.59–93.31]	88.72 [87.72–89.72]	67.33 [65.38–69.28]	77.16 [75.88–78.44]	NA	76.95 [75.62–78.28]	85.41
HLA-EMMA	91.18 [90.38–91.98]	81.54 [80.16–82.92]	82.91 [81.21–84.61]	NA	73.31 [71.72–74.90]	NA	67.97 [66.50–69.44]	79.38
HLA-Upgrade FR	85.83 [84.53–87.13]	87.02 [85.53–87.13]	82.33 [80.95–83.71]	NA	73.89 [72.40–75.38]	NA	76.11 [74.64–77.58]	81.04
HLA-Upgrade EU	93.22 [92.43–94.01]	94.76 [94.07–95.45]	90.77 [89.91–91.63]	NA	80.82 [79.64–82.00]	NA	82.91 [81.76–84.06]	88.50
HaploSFHI	<b>93.82 [93.28–94.36]</b>	<b>96.38 [95.95–96.81]</b>	<b>91.68 [91.06–92.30]</b>	<b>83.89 [82.17–85.61]</b>	<b>82.00 [80.85–83.15]</b>	<b>88.91 [88.02–89.80]</b>	<b>94.76 [94.09–95.43]</b>	<b>91.73</b>

Note: Averages are computed on loci HLA-A, -C, -B, -DRB1 and -DQB1. Bold font depicts the most accurate tool for each locus.

TABLE 3 B Percentages (and 95% CIs) of typings correctly imputed at the second field resolution level from the Nizhny Novgorod, Russia NGS test set ( $n = 1510$ ).<sup>14</sup>

	HLA-A	-C	-B	-DRB3/4/5	-DRB1	-DQA1	-DQB1	Average
HaploStats CAU	96.69 [96.03–97.32]	94.37 [93.54–95.17]	92.45 [91.49–93.38]	NA	85.36 [84.07–86.62]	NA	83.41 [82.09–84.70]	90.46
HaploStats EURCAU	95.79 [95.00–96.56]	93.48 [92.55–94.37]	91.75 [90.73–92.75]	NA	84.54 [83.18–85.89]	NA	82.62 [81.23–83.97]	89.64
HLA-EMMA	95.86 [95.17–96.56]	75.26 [73.74–76.82]	87.42 [86.26–88.58]	NA	77.28 [75.79–78.77]	NA	72.25 [70.66–73.81]	81.61
HLA-Upgrade FR	91.42 [90.10–92.68]	90.07 [88.71–91.39]	86.46 [85.00–87.91]	NA	81.49 [79.90–83.05]	NA	81.39 [79.80–82.95]	86.17
HLA-Upgrade EU	<b>96.82 [96.13–97.45]</b>	95.43 [94.67–96.16]	<b>92.58 [91.62–93.54]</b>	NA	<b>86.95 [85.70–88.18]</b>	NA	86.42 [85.20–87.62]	91.64
HaploSFHI	96.49 [95.79–97.15]	<b>97.25 [96.66–97.85]</b>	89.60 [88.51–90.66]	NA	85.50 [84.17–86.79]	NA	<b>95.13 [94.27–95.83]</b>	<b>92.79</b>

Note: Averages are computed on loci HLA-A, -C, -B, -DRB1 and -DQB1. Bold font depicts the most accurate tool for each locus.

TABLE 3 C Percentages (and 95% CIs) of typings correctly imputed at the second field resolution level from the Hammersmith Histocompatibility and Immunogenetics, London, United Kingdom NGS test set ( $n = 361$ ).

	HLA-A	-C	-B	-DRB3/4/5	-DRB1	-DQA1	-DQB1	Average
HaploStats CAU	93.35 [91.27–95.29]	92.52 [90.44–94.60]	93.91 [91.83–95.84]	NA	81.99 [79.09–84.76]	NA	81.58 [76.67–84.49]	88.67
HaploStats EURCAU	92.52 [90.30–94.60]	91.55 [89.20–93.77]	92.38 [90.03–94.60]	NA	80.61 [77.56–83.52]	NA	80.33 [77.15–83.38]	87.48
HLA-EMMA	91.41 [89.34–93.35]	81.44 [78.53–84.21]	85.18 [82.55–87.67]	NA	76.87 [73.55–80.06]	NA	74.24 [70.91–77.56]	81.83
HLA-Upgrade FR	88.50 [85.46–91.41]	88.64 [85.60–91.55]	87.26 [84.07–90.30]	NA	78.53 [75.07–81.99]	NA	81.72 [78.25–85.04]	84.93
HLA-Upgrade EU	93.77 [91.69–95.57]	96.54 [95.15–97.78]	<b>94.74 [92.94–96.54]</b>	NA	<b>84.76 [82.09–87.43]</b>	NA	86.57 [84.04–89.10]	91.00
HaploSFHI	<b>93.91 [92.11–95.71]</b>	<b>96.54 [95.15–97.78]</b>	92.52 [90.44–94.46]	NA	84.49 [81.79–87.19]	NA	<b>95.71 [94.04–97.33]</b>	<b>92.63</b>

Note: Averages are computed on loci HLA-A, -C, -B, -DRB1 and -DQB1. Bold font depicts the most accurate tool for each locus.



**TABLE 3 D** Percentages (and 95% CIs) of typings correctly imputed at the second field resolution level from the Scottish National Blood Transfusion Service Histocompatibility and Immunogenetics laboratory, Edinburgh, Scotland NGS test set ( $n = 504$ ).

	HLA-A	-C	-B	-DRB3/4/5	-DRB1	-DQA1	-DQB1	Average
HaploStats CAU	96.33 [94.94-97.62]	95.04 [93.65-96.33]	95.14 [93.65-96.53]	NA	84.82 [82.64-87.00]	0	81.05 [78.57-83.53]	90.48
HaploStats EURCAU	95.54 [93.95-97.02]	94.25 [92.66-95.73]	93.95 [92.16-95.54]	NA	82.84 [80.46-85.22]	0	79.56 [76.98-82.04]	89.23
HLA-EMMA	96.03 [94.74-97.22]	74.70 [72.02-77.28]	87.40 [85.22-89.48]	NA	77.78 [75.20-80.26]	0	76.29 [73.71-78.97]	82.44
HLA-Upgrade FR	90.28 [87.80-92.56]	91.27 [88.89-93.55]	89.58 [87.10-91.96]	NA	82.64 [79.86-85.32]	0	80.65 [77.78-83.43]	86.88
HLA-Upgrade EU	<b>96.43 [95.04-97.72]</b>	97.12 [96.03-98.12]	<b>95.44 [94.05-96.83]</b>	NA	87.40 [85.32-89.38]	0	85.12 [82.84-87.40]	92.30
HaploSFHI	96.13 [94.74-97.42]	<b>97.72 [96.83-98.61]</b>	94.54 [92.96-95.93]	NA	<b>88.00 [85.91-89.98]</b>	<b>94.94 [93.55-96.83]</b>	<b>95.63 [94.35-96.83]</b>	<b>94.40</b>

Note: Averages are computed on loci HLA-A, -C, -B, -DRB1 and -DQB1. Bold font depicts the most accurate tool for each locus. [Correction added on 20 October 2023 after first online publication: In Table 3D heading, n value has been updated to  $n = 504$  in this version].

**TABLE 3 E** Percentages (and 95% CIs) of typings correctly imputed at the second field resolution level from the Centro De Sangue E Transplantação Do Porto, Porto, Portugal NGS test set ( $n = 215$ ).

	HLA-A	-C	-B	-DRB3/4/5	-DRB1	-DQA1	-DQB1	Average
HaploStats CAU	94.19 [91.63-96.28]	94.19 [91.86-96.28]	90.00 [86.98-92.79]	NA	78.37 [74.65-82.09]	0	81.86 [78.14-85.35]	87.72
HaploStats EURCAU	92.79 [90.00-95.35]	93.49 [90.93-95.81]	88.84 [85.81-91.86]	NA	76.28 [72.33-80.00]	0	77.91 [73.72-81.86]	85.86
HLA-EMMA	93.95 [91.63-96.05]	83.26 [79.77-86.51]	79.77 [75.81-83.49]	NA	66.74 [62.33-71.16]	0	71.63 [67.21-76.05]	79.07
HLA-Upgrade FR	82.79 [78.14-87.21]	83.49 [78.84-87.91]	78.60 [73.72-83.26]	NA	70.47 [65.58-75.12]	0	75.35 [70.23-80.01]	78.14
HLA-Upgrade EU	<b>94.19 [91.86-96.51]</b>	94.88 [92.78-96.98]	<b>90.00 [86.98-92.79]</b>	NA	<b>80.70 [76.85-84.55]</b>	0	84.88 [81.40-88.37]	88.93
HaploSFHI	93.72 [91.40-95.81]	<b>95.81 [93.95-97.67]</b>	88.60 [85.58-91.40]	NA	78.60 [74.76-82.44]	<b>91.40 [88.60-93.95]</b>	<b>94.42 [92.09-96.51]</b>	<b>90.23</b>

Note: Averages are computed on loci HLA-A, -C, -B, -DRB1 and -DQB1. Bold font depicts the most accurate tool for each locus.

TABLE 3 F Percentages (and 95% CIs) of typings correctly imputed at the second field resolution level from intermediate resolution inputs.

	HLA-A	-C	-B	-DRB3/4/5	-DRB1	-DQA1	-DQB1	Average
HaploStats CAU	91.88 [89.22-94.40]	73.25 [69.75-76.61]	92.58 [89.92-94.96]	NA	91.60 [88.94-94.12]	NA	85.43 [82.35-88.38]	86.95
HaploStats EURCAU	86.13 [82.63-89.50]	67.79 [64.01-71.57]	86.13 [82.63-89.50]	NA	85.57 [82.07-88.94]	NA	79.27 [75.63-82.91]	80.98
HLA-EMMA	NA	NA	NA	NA	NA	NA	NA	
HLA-Upgrade FR	NA	NA	NA	NA	NA	NA	NA	
HLA-Upgrade EU	92.72 [90.20-94.96]	74.23 [70.87-77.59]	93.42 [90.90-95.66]	NA	92.44 [89.92-94.82]	NA	86.13 [83.19-88.94]	87.79
HaploSFHI	<b>97.48 [96.36-98.60]</b>	<b>74.51 [71.29-77.73]</b>	<b>97.90 [96.78-98.88]</b>	NA	<b>98.60 [97.62-99.44]</b>	NA	<b>99.58 [99.02-100.00]</b>	<b>93.61</b>

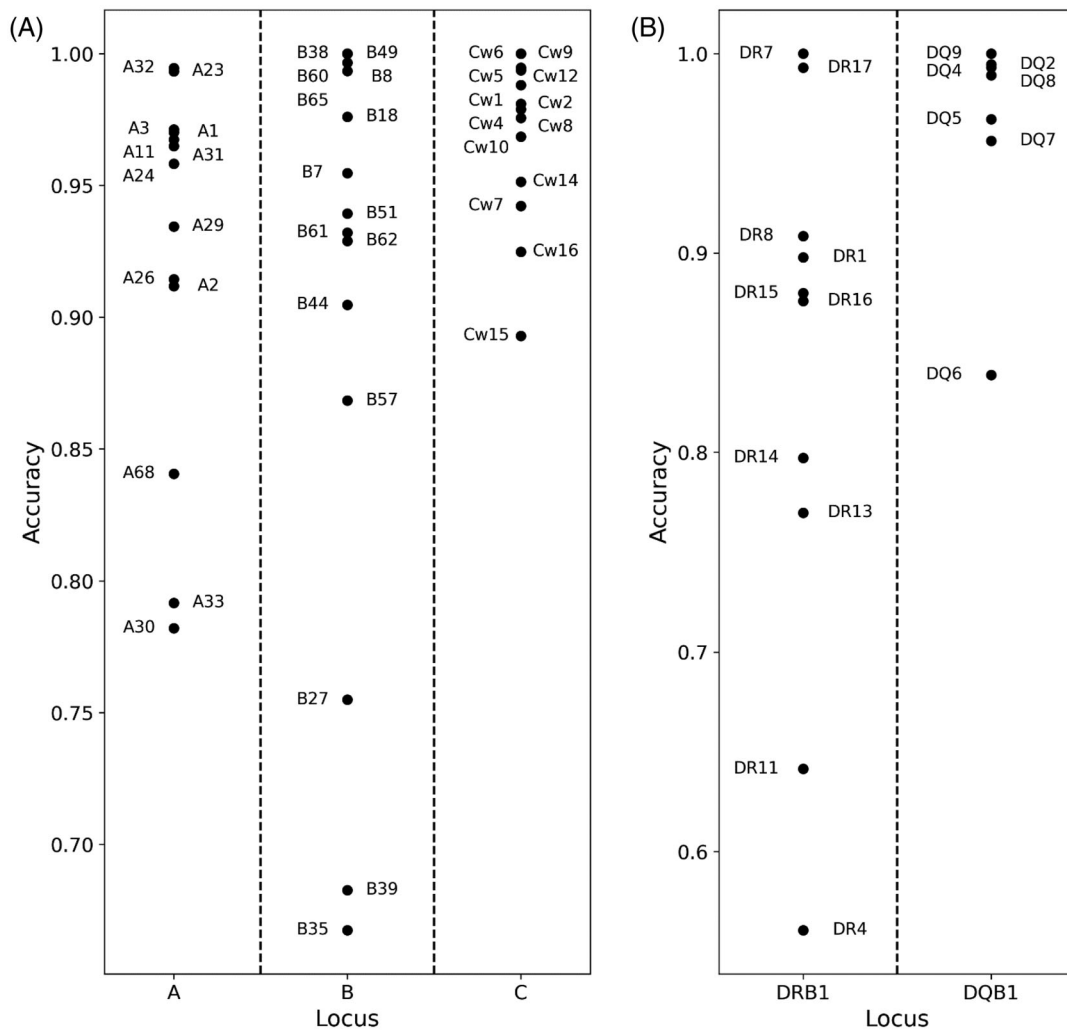
Note: French test set ( $n = 357$ ). Averages are computed on loci HLA-A, -C, -B, -DRB1 and -DQB1. Bold font depicts the most accurate tool for each locus.

typing can be found in Figure 2A,B for HaploSFHI and in Figures S1–S3 for HaploStats CAU, HLA-EMMA, and HLA-Upgrade EU. Serological groups that were too uncommon in the test set (less than 100 occurrences) were removed from the figures.

### 3.4 | Eplet analysis of imputation errors

For the eplet analysis, only HaploStats CAU, HLA-Upgrade EU, and HaploSFHI were considered and only with the FR-A test set. All the test set *predicted* second field typing(s) were known by the epitope registry database. However, 31 out of the 2145 of the test set, *true* second field typing(s) were not, for example, A\*30:80. Therefore, since the computation of this second metric was not feasible for these individuals, they were not considered for this analysis. Furthermore, 10 individuals of the 2114 remaining were removed as neither HaploStats CAU nor HLA-Upgrade EU returned any result. Indeed, keeping them would have led to very high numbers of FN eplets hugely skewing the average to the disadvantage of these two tools. HaploSFHI provided the least average FN eplets (Table 4A), except for class I eplets for which HLA-Upgrade EU performed better. HaploSFHI provided the least average FP eplets overall (Table 4B). HaploStats appeared as the least efficient in all settings.

We then investigated, using HaploSFHI imputations, which serological level input typings led to the most FN/FP eplets. DR4 was the least accurately imputed at the second field level and unsurprisingly led to the highest eplet inaccuracy. However, depending on the haplotype, an imputation tool might have its two most likely second field typing(s) as quite similar eplet-wise or, rather, two very different second field typing(s) eplet-wise. This information can help guide the practitioner as to whether a higher-resolution typing technique may bring useful information. Figure 3A (with more details in Table S11) reports the FN and FP DR4 eplets and assesses whether an imputation tool's first two choices are similar. As an example, a typing where the first two choices are DRB1\*04:03 and DRB1\*04:04 only displays one eplet difference, amino acid 74A being present only on DRB1\*04:04 and 74E being present only on DRB1\*04:03. In contrast, for DRB1\*04:02 and DRB1\*04:05, eight eplets are present only on DRB1\*04:02 (57S, 67LQ, 70QT, 70Q, 70QRA, 70QA, 71R, 86G) and eight eplets are present only on DRB1\*04:05 (57DA, 57D, 67I, 70DA, 70D, 71E, 85VV, 86 V). Figure 3B shows that DQ6 behaves like DR4, with DQB1\*06:04 and DQB1\*06:09 being very similar and DQB1\*06:04 and DQB1\*06:01 very different (more details in Table S12). In class I, B35 exhibits the most complexity, and the FN/FP eplets heatmap can be found in Figure 3C (more details in Table S13). The number of times where HaploSFHI predicted the typing on the



**FIGURE 2** (A) Assessing HaploSFHI accuracy for each class I input typing. Only serological groups that appeared at least 100 times in the testing set are displayed on the plot. (B) Assessing HaploSFHI accuracy for each class II input typing. Only serological groups that appeared at least 100 times in the testing set are displayed on the plot.

	Class I	Class II	Total
HaploStats CAU	0.36 [0.32-0.40]	1.71 [1.58-1.84]	2.07 [1.92-2.22]
HLA-Upgrade EU	<b>0.32 [0.28-0.36]</b>	1.14 [1.05-1.23]	1.46 [1.36-1.56]
HaploSFHI	0.35 [0.31-0.39]	<b>1.01 [0.92-1.10]</b>	<b>1.36 [1.26-1.46]</b>

Note: Bold font depicts the most accurate tool for each locus.

	Class I	Class II	Total
HaploStats CAU	0.37 [0.33-0.41]	1.74 [1.60-1.88]	2.11 [1.96-2.26]
HLA-Upgrade EU	0.36 [0.32-0.40]	1.09 [1.00-1.19]	1.45 [1.35-1.55]
HaploSFHI	<b>0.35 [0.31-0.39]</b>	<b>1.04 [0.95-1.13]</b>	<b>1.39 [1.29-1.49]</b>

Note: Bold font depicts the most accurate tool for each locus.

**TABLE 4A** Average number of FN eplets (and 95% CIs) when extrapolated at the second field resolution level from the HaploSFHI test set ( $n = 2104$ ).

**TABLE 4B** Average number of FP eplets (and 95% CIs) when extrapolated at the second field resolution level from the HaploSFHI test set ( $n = 2104$ ).

y-axis instead of the one on the x-axis is also included in Figure 3A–C. Serological-level homozygotes were not included because it could not clearly be determined what

the misprediction was. Interloci eplets were not included because other class II loci could compensate for the eplet misprediction. Besides, because of LD, some interloci eplets

are more likely to be compensated than others, making the analysis too convoluted.

Computing FN and FP eplets for all possible pairs of alleles enabled us to identify second field typing(s) for which misprediction would not result in any eplet error. This identifies groups of alleles, reported in Table S10, that should not trigger B cell responses as B cell activation requires a nonself eplet.<sup>17</sup>

### 3.5 | Availability and use of HaploSFHI

HaploSFHI is freely available at [www.sfhitools.fr/HaploSFHI](http://www.sfhitools.fr/HaploSFHI). Incomplete inputs (e.g., no serological level HLA-C), typings of intermediate-resolution transformed in the list of ambiguities (e.g., obtained with real-time PCR), and mixed inputs (e.g., one or several loci already known at the second field level) can also be imputed. For class I imputations (HLA-A, -C, and -B), at least first field (serological level or supertypes) HLA-A and -B should be inputted. For class II imputations (HLA-DRB3/4/5-DRB1, -DQA1, and -DQB1), at least first field (serological level or supertypes) HLA-B, -DRB1, and -DQB1 should be inputted. HLA-DPA1 imputation results based on HLA-DPB1 are available in the documentation of the website.

## 4 | DISCUSSION

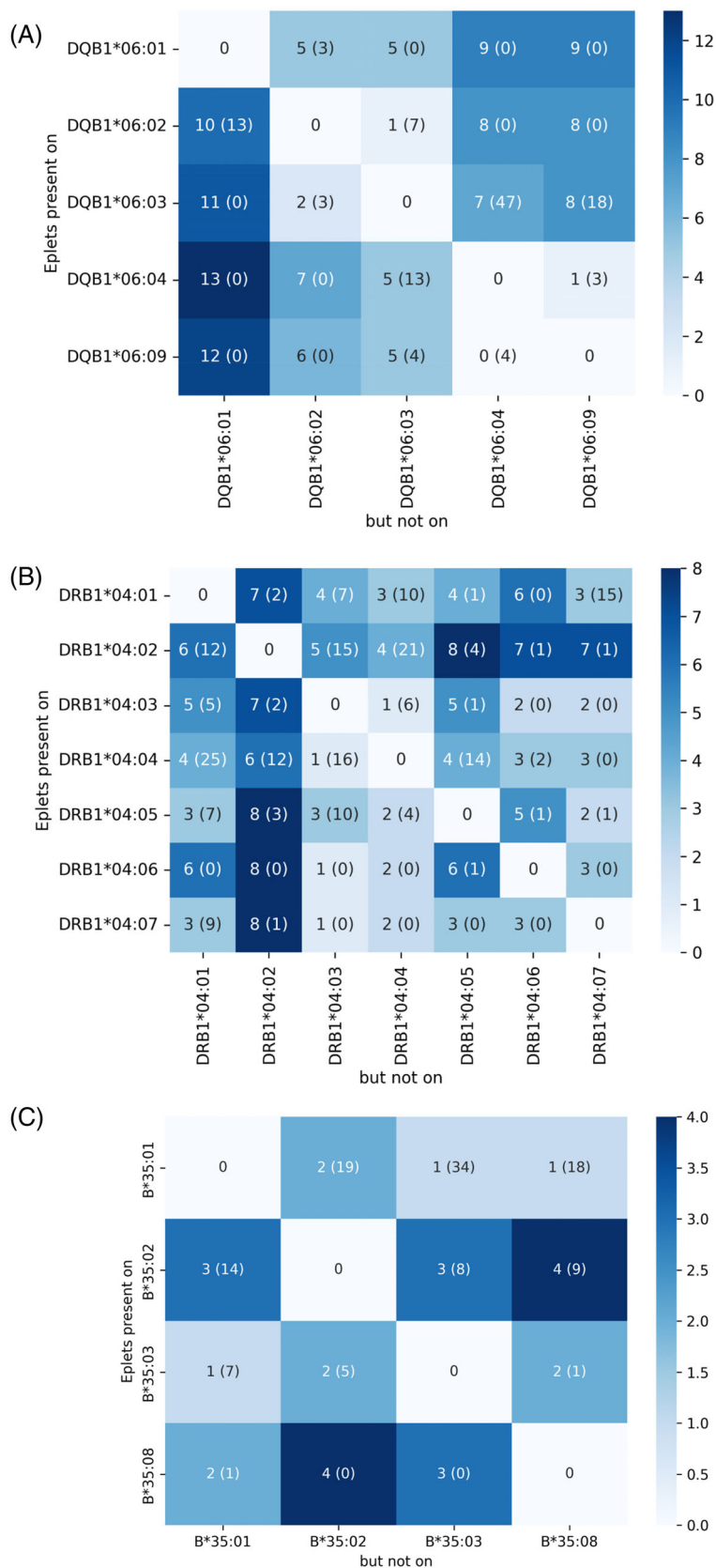
The need for second field typing(s) without ambiguity combined with the imprecision of the current typing imputation tools is a recurrent concern.<sup>10,18,19</sup> This educated guesswork, imputation, is commonly used in routine work and has been employed in significant studies involving HLA molecular mismatch analysis so far.<sup>7</sup> We report the development and validation of HaploSFHI, a new HLA typing imputation tool. Our findings demonstrate that HaploSFHI outperforms existing tools on common loci when applied to French and European/Caucasian datasets. HaploSFHI also includes loci not (-DQA1, -DPA1, -DPB1) or rarely (-DRB3/4/5, only by HaploStats) considered so far, despite their known clinical relevance in transplantation. We focused on a training set composed exclusively of local NGS typings from residents of France, showing that including typings from a wider origin is not necessary for an algorithm to learn the imputation process.

### 4.1 | Factors impacting the performance of the predictions

All three tools using LD displayed much better results than the simpler approach of choosing the most frequent second

field typing(s) associated with the serological level typing input, as expected given the already well-established non-random association of HLA-A, -C, -B, -DRB3/4/5, -DRB1, -DQA1, and -DQB1 alleles. For instance, the frequent B44 serological group average imputation accuracy is around 50% with HLA-EMMA (Figure S2A) but around 90% with other tools (Figure 2A; Figures S1A and S3A). This highlights the importance of LD when more than 1 s field typing is frequent. The relatively low scores of HLA-Upgrade FR compared with HLA-Upgrade EU are likely due to the relatively small French training set used by Geffard et al.<sup>12</sup> The knowledge of the HLA allelic polymorphism that was available when the tools were developed also impacts the tool's performance. Indeed, alleles that were mistyped in the past, for example, because a sequence polymorphism was located in a region of the gene that was not analyzed by the techniques and reagent kits available, may actually be the most frequent of an antigenic series. This leads to a high frequency of mispredictions nowadays as typing technologies have evolved towards deeper analysis of HLA genes. For example, HaploStats and HLA-Upgrade errors are mainly due to typings, such as DRB1\*14:54 and DQB1\*02:02, which are, respectively, predicted as DRB1\*14:01:01G and DQB1\*02:01:01G. This is evident when comparing Figure 2A,B with Figure S1. When considering the representative example of the DQ2 antigen, its accuracy significantly decreases from nearly 100% when training with NGS typing data to less than 50% when training without NGS typing training data. This is consistent with the fact that DQB1\*02:01 is found in 10.32% of the individuals in our database and DQB1\*02:02 in 11.28%, both representing >99% of the DQ2 encountered. Since LD for DRB1-DQB1\*02 haplotypes is straightforward with DR17-DQ2 haplotypes bearing DBQ1\*02:01, and DR7-DQ2, DR4-DQ2, DR9-DQ2 haplotypes bearing DQB1\*02:02, a data set such as ours allows getting close to 100% on DQ2 average accuracy. Similarly, DR14 average accuracy is near 80% with NGS typing in the training set but drops to 20% for tools not differentiating DRB1\*14:01 from DRB1\*14:54 and always predicting DRB1\*14:01. This is coherent with the fact that DRB1\*14:01 is found in around 0.37% of the individuals of our database and DRB1\*14:54 in 2.76%, both representing 82% of the DR14 encountered. Interestingly, yet logically, as shown in Figure S2B, HLA-EMMA, being more recent than HLA-Upgrade and HaploStats, has a good average accuracy on DR14 as it systematically returns DRB1\*14:54 for a DR14 input.

All these limitations and our findings support that a recent training set, obtained with up-to-date techniques and especially NGS approaches performed on a maximum of genes displaying a clinical interest, offers better performance. This recent training set does not have to be as large as those used by older tools.



**FIGURE 3** (A) Number of eplets present on the y-axis DR4 allele but not on the x-axis DR4 allele (gray scale). Numbers between brackets are the error frequency in the HaploSFHI testing data set (number of times a y-axis DR4 allele was predicted instead of an x-axis DR4 allele—out of 526 times). (B) Number of eplets present on the y-axis DQ6 allele but not on the x-axis DQ6 allele (gray scale). Numbers between brackets are the error frequency in the HaploSFHI testing data set (number of times a y-axis DQ6 allele was predicted instead of an x-axis DQ6 allele—out of 706 times). (C) Number of eplets present on the y-axis B35 allele but not on the x-axis B35 allele (gray scale). Numbers between brackets are the error frequency in the HaploSFHI testing data set (number of times a y-axis B35 allele was predicted instead of an x-axis B35 allele—out of 364 times).

As expected, the performance of HaploSFHI was notably higher for all loci on the French validation cohorts, considering its training was conducted on a

French data set. Furthermore, our study reveals that an NGS training set leads to excellent performance even on data sets derived from neighboring populations that were

not originally included in the training set. Although HaploSFHI's average accuracy is significantly higher on these foreign datasets, HaploSFHI does not show significant improvement (nor degradation) on the HLA-A, -B, and HLA-DRB1 loci compared with other tools.

A training set relying exclusively on NGS typing data does not address all the imputation shortcomings. All tools revealed subpar performance for DRB1 compared with other loci. The explanation relies mainly on a few serological level typings for which the second field is difficult to predict due to the presence of several frequent DRB1 alleles hardly distinguishable even when using LD. For instance, DR13 (14% of the typings) encompasses three frequently occurring alleles: DRB1\*13:01 to 13:03, present in 7.15%, 4.54%, and 1.39% of our local population, respectively, and DRB1\*13:04 and DRB1\*13:05 found in 0.29% and 0.19%, respectively, and although LD retrieves two common haplotypes, this is not sufficient: DR13 associated with DQ6 is usually \*13:01 or \*13:02, distributed 60%/40% in our database and DR13 associated with DQ7 is usually \*13:03, \*13:04 or \*13:05, distributed 75%/15%/10%. Considering LD with HLA-B helps, but remains insufficient to reach the accuracy of other loci.

## 4.2 | Eplet analysis

Incorrect prediction of a second field typing(s) is associated with the risk of eplet identification errors and therefore errors in assessing donor/recipient eplet compatibility. The performance of HaploSFHI tended to be higher than that of HLA-Upgrade EU and HaploStats CAU. Although a correlation is expected between second field prediction error and eplet mismatch error at the level of a full typing or of a population of subjects, some second field typing(s) are frequently incorrectly predicted without erroneous eplets repertoires, as distinct second field alleles may be very close in amino acid sequence for antibody-accessible regions of the molecule. To go further, one may remember that the imputation tools provide additional second field results besides the most likely one (except HLA-EMMA, see Materials and methods section). Therefore, the second choice output for a given serological typing may be identical in terms of eplets to the most likely second field typing(s) result. In this case, the immunological risk caused by an imputation error becomes very low. Overall, imputation into second field resolution level may not be the ultimate objective of such tools if the purpose is to measure donor/recipient incompatibility for the immune system. Indeed, for the B cell response, tools capable of predicting eplet repertoires rather than second field typing(s) prediction may represent a satisfying alternative for clinical application.

## 4.3 | Perspectives

Overall, the present work shows that a high but not necessarily enormous number of local well-documented HLA typings, reaching the second field level of resolution without allele ambiguities, that is, using a sequencing approach, is a suitable way to generate a powerful tool to impute typings of initially insufficient resolution. It would be interesting to repeat our local initiative in other countries in different areas of the globe to validate our findings. For DPB1 and DPA1, imputation remains feasible only if first field DPB1 is known, due to the very weak LD that exists between the DP locus and the other HLA genes (results not shown). Increasing the number of local typings might be the best option to identify class II haplotypes encompassing DPB1 and DPA1, if they do exist. Similar findings would probably be obtained with our tool regarding a T-cell immune response metric, which depends on the antigenic peptide binding groove, the area of the HLA molecule showing an even larger polymorphism than the regions accessible to antibodies covered by the eplet approach.

### AUTHOR CONTRIBUTION

*Study conception and design:* Romain Lhotte, Jean-Luc Taupin. *Data collection:* Romain Lhotte, Cédric Usureau, Debora Jorge-Cordeiro, Jérémy Siemowski, Jean-Luc Taupin. *Analysis and interpretation of results:* Romain Lhotte, Véronique Letort, Jean-Luc Taupin. *Draft manuscript preparation:* Romain Lhotte, Véronique Letort, Lionel Gabet, Cédric Usureau, Paul-Henry Cournede, Jean-Luc Taupin. All authors reviewed the results and approved the final version of the manuscript.

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### CONFLICT OF INTEREST STATEMENT

The author declares no conflict of interest regarding the content and findings presented in this article.

### DATA AVAILABILITY STATEMENT

Data available in Supplementary Tables S1–S9 are also provided in a single Comma-Separated Files (.csv). For anonymization purposes, the data are supplied in the form of frequencies of second field typing(s) for each gene, rather than complete second field typing(s) for all individuals.

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## SUPPORTING INFORMATION

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

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