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PCR optimization for buccal swab-derived samples: overcoming sporadic inhibition with bovine serum albumin

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Abstract

Background Buccal swabs are widely used as a non-invasive method for genomic DNA collection in large-scale genotyping studies. However, the sporadic presence of PCR inhibitors within these samples may hinder PCR amplification and affect assay reliability. This study investigates the incorporation of bovine serum albumin (BSA) into the PCR reaction mixture as an additive to counteract PCR inhibition.

Results In our high-throughput setting, the incorporation of BSA significantly improved robustness, lowering failure rates to 0.1% in subsequent routine operation across 1,000,000 buccal swab samples. Despite minor challenges with foaming during automated liquid handling, no detrimental effects on PCR performance were observed.

Conclusion These results underscore the efficacy of BSA in improving PCR robustness, enhancing the reliability of high-throughput molecular diagnostic assays.

Keywords Buccal swab-derived DNA, PCR, PCR inhibitors, BSA, HLA genotyping

Introduction

Buccal swabs have emerged as a prevalent method for DNA collection in both clinical and research contexts due to their non-invasive nature, ease of use, and cost-effectiveness [1]. They allow for the collection of epithelial cells from the buccal mucosa for a range of DNA-based analyses, including genotyping [2], forensic examination [3], and diagnostic assays [4]. The DNA extracted from buccal swabs is generally of high quality [5], especially when state-of-the-art purification techniques are employed. While traditional methods like phenol–chloroform often introduce PCR inhibitors [6], modern techniques such as magnetic bead-based and silica membrane technologies streamline the extraction process and improve both purity and scalability [7].

Despite the general reliability of DNA extracted from buccal swabs, occasional PCR inhibition remains a challenge. This problem arises from unidentified inhibitory substances that can interfere with polymerase activity, resulting in diminished amplification efficiency or complete reaction failure [8]. In buccal swab-derived DNA extracts, inhibitory substances may derive from cosmetic products, in particular lip balm, lip gloss, or lipstick that may get collected with the swab unintentionally. In particular polyanionic polymers (e.g. alginates, polyacrylates) might copurify with the DNA and inhibit the PCR reaction.

One promising approach to mitigate PCR inhibition involves incorporation of bovine serum albumin (BSA)

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into the PCR reaction mixture. BSA has been shown to non-specifically bind inhibitory substances, thereby protecting DNA polymerases and enhancing PCR efficiency [9]. Although previous studies have highlighted BSA's potential to improve PCR performance in the presence of inhibitors, they have primarily focused on DNA extracted from various environmental or microbial sources. Furthermore, due to the limited sample sizes of these proof-of-concept studies [10–14] it remained questionable if potential detrimental effects might offset the beneficial aspects in a high-throughput setting.

In this study, we aim to extend these preliminary findings by assessing the impact of BSA on PCR inhibition across a significantly larger and more diverse cohort of 1,000,000 human buccal swab samples. By analyzing an extensive sample set, we seek to provide comprehensive evidence of BSA's effectiveness in overcoming PCR inhibition, thus supporting its broader application in molecular diagnostics and other contexts where PCR inhibition may be problematic.

Material and methods

Samples and DNA preparation

The buccal swab samples analyzed in this study were processed between May 2021 and December 2024. Buccal swabs were collected from new potential hematopoietic stem cell donors in Germany, Poland, the United Kingdom, the United States, and Chile in accordance with the consent forms signed at recruitment [15]. All samples, irrespective of their country of origin, were processed with the same automated workflow with samples from different countries often being processed jointly on one plate. Buccal swab sampling was performed by the donors using "FLOQSwab™ hDNA free" (Copan Italia Spa, Brescia, Italy) according to instructions which state to "rub the buccal swab in a circular motion across the inside of one cheek for 1 min" followed by 2 min of ambient drying. Subsequently, swabs were placed in a paper envelope and transported and stored at ambient temperature for up to 6 weeks. DNA extraction was carried out using an automated process with the magnetic bead-based "chemagic™ DNA Buccal Swab Kit special" (Revvity (Germany) GmbH, Hamburg, Germany), with DNA eluted in 100 µL of elution buffer (10 mM Tris–HCl, pH 8.0). DNA concentrations were measured by a quantitative fluorescence assay based on SYBR Green I (Biozym, Hessisch Oldendorf, Germany) using the TECAN infinite 200Pro (Tecan, Männedorf, Switzerland) plate reader. Extracted DNA concentrations ranged from 2 to 180 ng/µL. Neither DNA isolation nor DNA quantification as applied is specific for human DNA. Therefore, it is expected that mucosal bacterial DNA is partly contributing to the total DNA [16]. For downstream applications, samples were diluted with water according to their initial DNA concentrations, following a dilution factor of 1:2.6 for concentrations between 2 and 20 ng/µL, and 1:7.8 for concentrations ranging from 20 to 180 ng/µL.

PCR amplification

PCR primers were designed to target the exons 2 and 3 of human leukocyte antigen (HLA)-A, -B, -C, -DPA1, -DPB1, -DQA1, -DQB1, -DRB1, and -DRB3, -DRB4, and -DRB5 [17]. The resulting amplicons ranged in size from ~ 300 bp to ~ 450 bp. PCR amplification was performed using the Roche Fast Start Kit (Roche Diagnostics GmbH, Mannheim, Germany) following the manufacture's protocol. Briefly, the PCR reaction mixture included 2 µL template DNA, 0.8 µL 10 × FastStart High Fidelity Reaction Buffer without $MgCl_2$, 0.2—1.1 µM target-specific primer mix, 2 mM $MgCl_2$, 200 µM Nucleotide Mix, 0.4 µL DMSO, 0.4 U Fast Start Taq Polymerase, and PCR grade water to a final volume of 8 µL. BSA (Fatty Acid-Free, Nuclease- and Protease-Free, Merck, 126,609) was added to a final concentration of 0.1 µg/µL starting in May 2023; prior to this, no BSA was included in the reaction mix. Selected PCR products were separated by agarose gel electrophoresis and analyzed under UV light. A 100 bp-DNA-ladder (Carl Roth GmbH + Co.KG, Karlsruhe, Germany) (100 bp to 1000 bp with enhanced 500 bp fragment) served as a molecular size standard.

High-throughput HLA genotyping workflow

High-throughput HLA genotyping was performed as previously described [2, 17]. All targets were amplified in primary PCR reactions using 384-well plates. Amplification products were pooled sample-wise and indexed with molecular identifiers by a second PCR. 384 samples were pooled and subsequently purified using SPRIselect Beads (Beckman Coulter, Brea, USA). DNA quantification was performed by qPCR (Library Quant Illumina Kit, KAPA Biosystems, Boston, USA and ABI-StepOnePlus, Thermo Fisher, Carlsbad, USA). The purified amplicon pools were mixed in equimolar amounts and sequenced according to Illumina's protocol on a NovaSeq 6000 instrument (Illumina, San Diego, USA) [2]. Data analysis employed neXtype, an in-house developed software tool [17] with the IPD-IMGT/HLA database as reference [18]. Throughout this manuscript we define the failure rate as the proportion of samples where amplification failed for six or more loci, resulting in insufficient reads for genotyping.

Results

In 2013, we introduced a high-throughput HLA genotyping workflow [17], employing DNA isolation, HLA locus-specific DNA amplification, and Illumina-platform-based sequencing. This workflow allows cost-efficient donor genotyping across key HLA loci from buccal swab samples. As part of our continuous quality assessment, we

monitor the failure rate, which is defined as the proportion of samples in which multiple loci cannot be analyzed due to amplification failures. From May 2021 to September 2022, we observed average failure rates around 0.3%. However, starting in October 2022, failure rates increased considerably reaching up to 2% (Fig. 1a, Suppl. Table 1). A breakdown of failure rates by country revealed that primarily samples from Germany were affected, while samples from Poland, UK, USA, and Chile remained largely unaffected (Fig. 1b), suggesting that variations in the sample characteristics, rather than procedural issues, played a pivotal role.

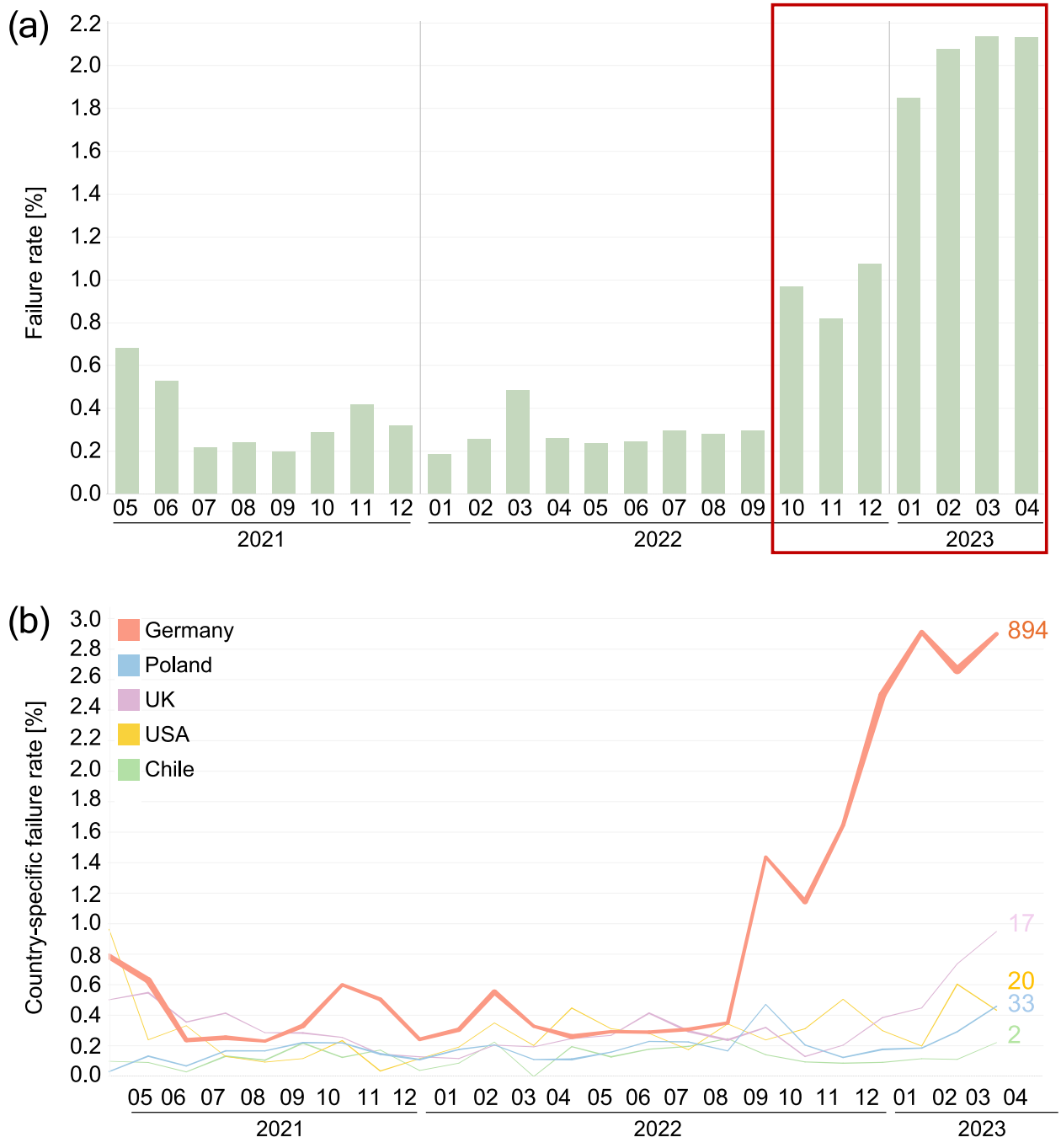


Fig. 1 HLA genotyping failure rates. **a** Between May 2021 and April 2023, average HLA genotyping failure rates for buccal swab-derived samples ranged from 0.2% (80 failed samples out of 40,775 in September 2021) to 2.14% (1,728 failed samples out of 80,864 in March 2023, Suppl. Table 1). Starting in October 2022, failure rates increased for unknown reasons. **b** Comparison of country-specific failure rates between May 2021 and April 2023 demonstrated that primarily samples from Germany (orange) were affected. Failure rates for samples from Poland (blue), UK (purple), USA (yellow), and Chile (green) remained below 1%. The colored values represent absolute failure rates by country for April 2023

Next, we analyzed DNA concentration levels as potential cause of increased failure rates. DNA concentration levels remained largely stable over time with minimal fluctuations and a slight downtrend (Fig. 2a). Moreover, correlation analysis indicated that failure rates increased consistently across all DNA concentration ranges (Fig. 2b, Suppl. Table 1), indicating that a sample quality parameter independent of DNA concentration was presumably causing the increased failure rates.

Gel electrophoresis of selected PCR products revealed the primary HLA-specific PCR as the main source of genotyping failures (Fig. 3a, Suppl. Figure 1, Suppl. Table 2). To rule out technical issues affecting PCR amplification, we manually repeated the PCR reactions using the same samples and conditions, reproducing the initial observations (Fig. 3a, Suppl. Figure 1). The observed pattern of PCR failure and success was largely sample specific and consistent across the seven target specific PCR reactions with completely independent primer sets. However, several samples that failed to amplify under routine conditions yielded faint amplification signals upon manual processing. To investigate potential PCR inhibition by unknown substances, we diluted all samples in water at ratios of 1:40 and 1:80. Both dilutions restored PCR amplification across most HLA loci, although the 1:80 dilution resulted in a lower yield (Fig. 3b, Suppl. Figure 2). These results, along with the reproducibility of PCR failure and the fact that only samples from Germany were affected, indicated the presence of inhibitory substances compromising PCR efficiency. However, while DNA dilution restored amplification capability in the selected samples, this obviously does not serve as a general solution to the problem of inhibitory substances: samples with low DNA concentrations might get diluted beyond concentrations that are adequate for efficient amplification. Therefore, we sought an alternative method to mitigate PCR inhibition caused by unidentified inhibitory compounds. We explored the use of BSA, which is known for its high binding capacity to inhibitory substances. As depicted in Fig. 3c, incorporating 0.1 µg/µL BSA into the PCR mixture restored amplification of the five previously non-amplifiable samples (Suppl. Figure 3).

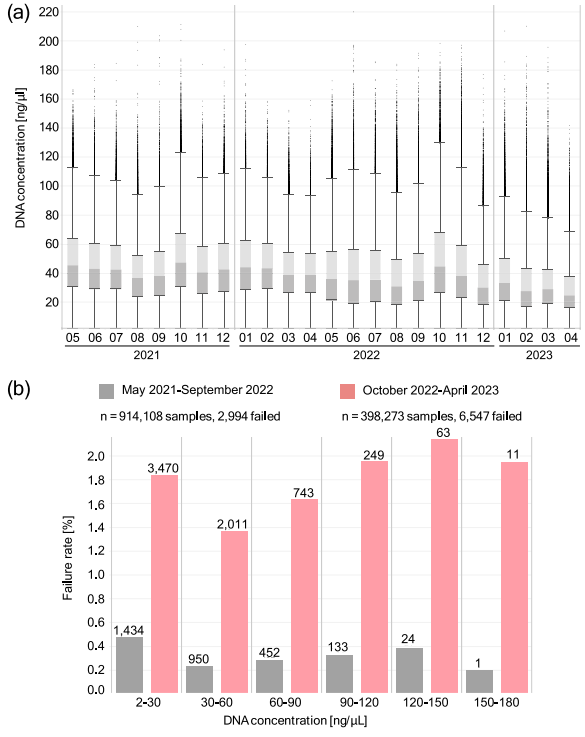


Fig. 2 Impact of DNA concentration on HLA genotyping failure rates. **a** Distribution of extracted DNA concentrations for samples from May 2021 to April 2023. **b** Genotyping failure rates stratified by extracted DNA concentration comparing time periods of low (prior October 2022, grey) and high failure rate (October 2022 to April 2023, red)

Following a series of promising small-scale experiments, we started the transfer to high-throughput routine operation. Initially, we observed increased foaming during the pipetting process when using automated liquid handlers. This issue was effectively managed through adjustments in liquid handling protocols and optimization of the automated pipetting systems. Starting in May 2023, BSA was systematically integrated into our high-throughput PCR processes. Over the following months, this modification was applied to a total of 1,000,000 samples. The implementation of BSA resulted in a markedly decreased failure rate of close to 0.1% (Fig. 4a). This dramatic improvement underscores BSA's effectiveness in mitigating PCR inhibition and enhancing overall assay robustness. It is noteworthy that the current failure rate of about 0.1% represents a marked improvement even when compared to the time in 2021/2022 before significant issues with PCR inhibitors became prominent. In addition, although increased failure rates were initially observed in German samples, the benefits of BSA were evident across samples from all geographic regions (Fig. 4b).

This strategic modification to our PCR workflow demonstrates BSA's potential in improving the reliability and efficiency of high-throughput PCR-based molecular diagnostic assays.

Discussion

DNA isolated from buccal swabs is typically of high quality. However, we experienced that the presence of PCR inhibitors may occasionally and to varying degrees impact amplification efficiencies. While these failures might be inconspicuous in small-scale studies, even failure rates of low single digit percentages may result in considerable additional costs and delays in high-throughput settings. Beginning in October 2022 we observed a five-fold increase in PCR failures from below 0.4% to 2%

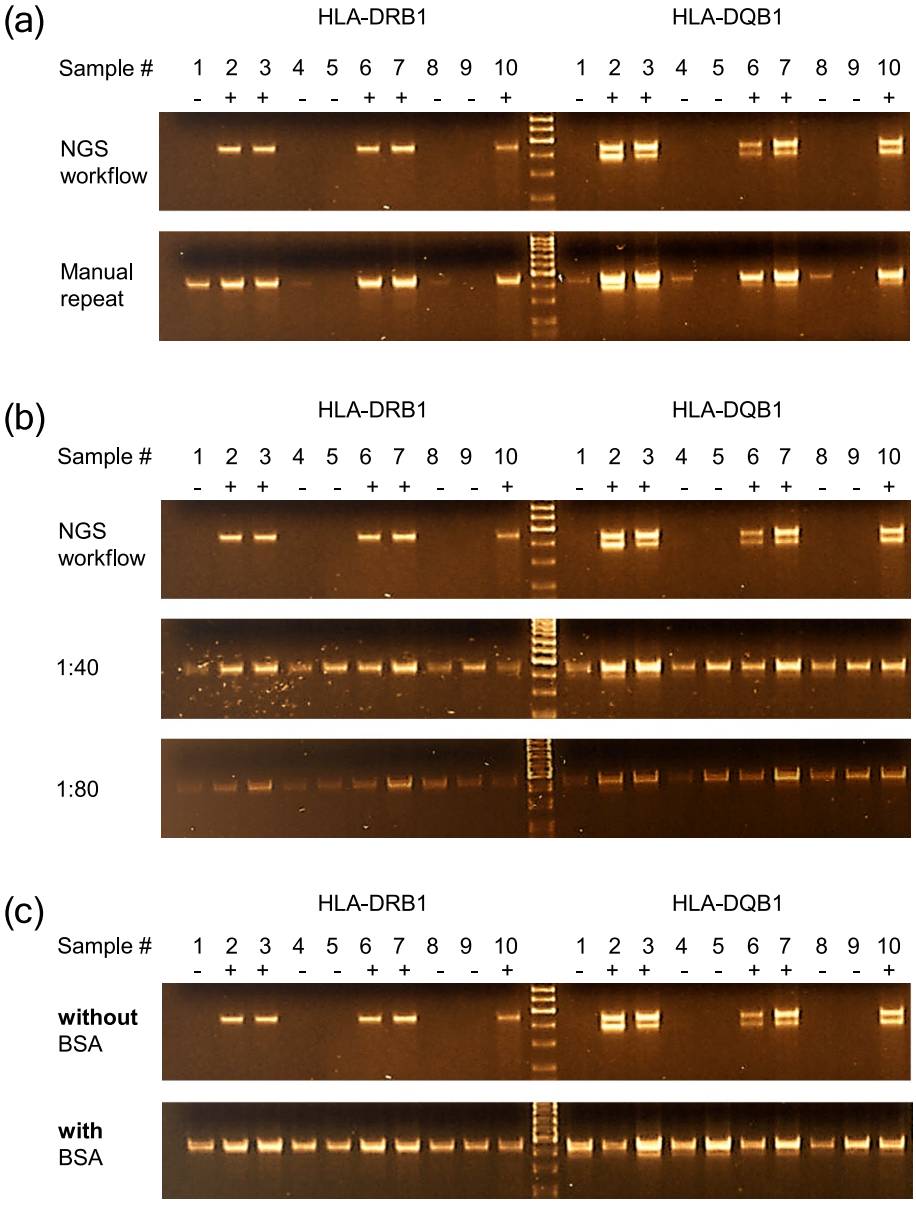


Fig. 3 PCR inhibition in affected samples and mitigating measures. Agarose gel electrophoresis of PCR products amplified with HLA-DRB1 or HLA-DQB1-specific primer sets. Ten samples were selected to represent those with successful (+) and failed (-) sequencing during routine operation (NGS workflow). **a** When repeating the PCR for these selected samples manually, the failure pattern is consistent with the results of the routine workflow. **b** Dilution of DNA with water (1:40 or 1:80) overcomes PCR inhibition. **c** Addition of 0.1 µg/µL BSA to the PCR reaction mixture overcomes PCR inhibition

in February 2023 in the absence of any relevant changes in the workflow and with no detectable process abnormalities. The concentration of the elevated failure rate to samples from Germany (Fig. 1b) strongly argues against process problems caused by some unintentional deviations during processing of the samples: First, samples are processed by sample receipt agnostic of the country of origin jointly in the same workflow with samples from different countries often ending up on the same plates. Second, failing samples were not clustered to few plates (that might have been subjected to some unintentional aberrant treatment) but distributed in a random fashion among tens of thousands of samples. Third, the same pattern of elevated failure rates specifically observed for samples from Germany continued over a period of seven months (Fig. 1b) and was obliviated only when we introduced BSA to the workflow. PCR experiments with affected samples showed that the failures are largely reproducible but may be overcome by dilution or addition of BSA (Fig. 3), strongly hinting at the presence of some unknown inhibitory substance or substances as the root cause for these elevated failure rates. Unfortunately, we do not have the means to identify the exact substance or substances causing the problems. We can

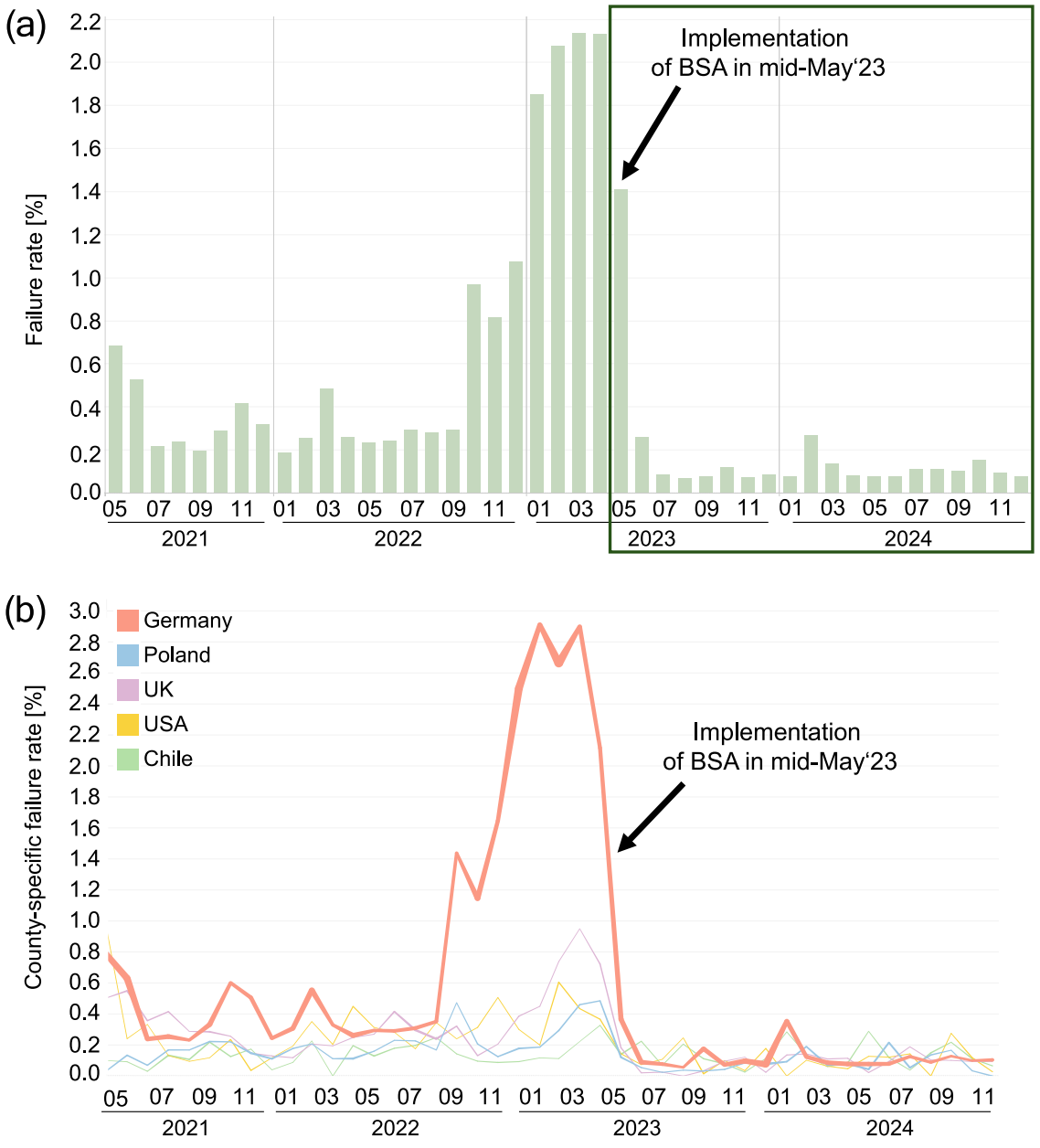


Fig. 4 Implementing BSA in high-throughput HLA genotyping from buccal swab samples reduces genotyping failure rates. **a** HLA genotyping failure rates for samples processed between May 2021 and December 2024. The introduction of BSA in mid-May 2023 reduced failure rates considerably. **b** Country-specific failure rates for samples from Germany (orange), Poland (blue), UK (purple), USA (yellow) and Chile (green)

only speculate that at that time some cosmetic product or product class was becoming quite popular in Germany that contained substances that copurify with DNA and have a strong inhibitory effect on PCR. To address the PCR inhibition, we incorporated BSA into the PCR mixture, as previous small-scale studies had indicated that BSA enhances amplification yield, particularly in the presence of inhibitors. This effect is likely due to BSA's broad absorption capabilities through both hydrophobic forces and ionic interactions [19, 20]. BSA has been reported to be effective across a range of concentrations [9]. The optimal concentration needs to be determined for each sample type, as it can vary depending on the nature of the inhibitors, the DNA isolation method, and the specific PCR components used. When establishing the addition of BSA for the buccal swab-derived samples, we evaluated BSA concentrations ranging from 0.05 µg/µL to 0.1 µg/µL without observing any differences, suggesting that the selected concentration was not a critical factor (data not shown). After incorporating BSA, we encountered increased foaming, attributed to BSA's polymeric and polyelectrolyte properties [21], necessitating

optimization of liquid handling protocols and automated pipetting systems. Once we addressed the foaming issue, no further adverse effects were detected in our high-throughput workflow. While previous small-scale studies had demonstrated the protective effect of BSA against inhibitory substances [10, 11, 20], the current high-throughput study provides robust evidence that BSA alleviates PCR inhibition without any detectable negative impact. This purely positive effect of BSA was demonstrated over an extended experimental period involving a total of 1,000,000 samples. A potential limitation of our study is the use of a specific Taq polymerase enzyme across all samples. However, it is important to note that previous studies have employed alternative enzymes distinct from ours, implying that the observed effects are not exclusively attributable to this polymerase but rather reflect a general characteristic of BSA.

Conclusion

In our high-throughput setting, BSA has proven to be a highly cost-effective additive with an expense of less than 1 Eurocent (€0.01) per sample. Its substantial impact on PCR robustness and the significant reduction in failure rates with DNA from buccal swab samples underscore BSA's value as a reliable and efficient additive for PCR in large-scale molecular diagnostic applications.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-12350-x.

Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Supplementary Material 4.

Supplementary Material 5.

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Authors' contributions

AK and KP designed and KP carried out the proof-of-concept experiments. DP, BJ, JG and YU drove the adaptation of the high-throughput workflow to the new protocol. BH, KH, JP and SB performed data analysis. ST and VL conceived the study and wrote the manuscript. AHS, AK and VL supervised the work. All authors read and approved the final manuscript.

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Data availability

The data of this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The buccal swabs analyzed in this study were genotyped at DKMS Life Science Lab as part of the registration process for potential stem cell donors. This is a worldwide accepted standard process to identify potential stem cell donors. The genotyping was within the scope of consent forms signed by all participants at the time of their registration with DKMS. No additional sampling was performed specifically for the study. All experiments were performed on stored samples to improve the quality of the processing. The study was conducted in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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