The Curious Case of Sporadic Amplification with the Same Manufacturer's Lot of Taq Polymerase

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Introduction

Next Generation Sequencing (NGS) has revolutionized the field of histocompatibility because it allows multiple patients to be rapidly typed at high resolution. However, when failure occurs during an NGS run, the impact is greater since many samples are affected, and the cost of reagents is high. Therefore, it is critical to quickly determine the cause and mitigate any future issues.

In 2024, we began experiencing sporadic and inconsistent failed amplifications in our NGS assay. Initially it was thought to be a technical error or poor quality of extracted DNA. Our laboratory amplifies 11 HLA Loci over three 96 well plates and the failures were not only sporadic between runs, but also within the set of amplification plates.

Two validated kit lots were in use, which made it more difficult to detect a pattern with the amplification failures. After establishing kit lot #581844 was the problem, identifying the kit component at the root of the failed amplifications became the priority.

Taq Polymerase Lot #581844

Shipment Date	QC Date	Quality Control (QC) Results
2/16/2024	02/22/2024	Passed
3/26/2024	03/28/2024	Failed (Issue unknown)
	04/01/2024	Failed (No correlation determined between failures)
	04/08/2024	Passed (Previous issue unresolved).
5/23/2024	05/23/2024	Passed (Low amp for DRB4 detected but was within acceptable QC range).

07/02/2024	Taq Polymerase Lot #575019 exhausted
07/05/2024	Taq Polymerase Lot #581844 routine use started.
07/08/2024-07/15/2024	Multiple failures at different loci detected over the course of six NGS runs.

Investigative Results

07/15/2024	Internal investigation determined Lot #581844 received 3/26/2024 and 5/23/2024 could not be used. Vendor contacted and was very responsive assisting with investigation
07/16/2024	ClinImmune provided vendor with gel from failed amplification (Fig. 1).

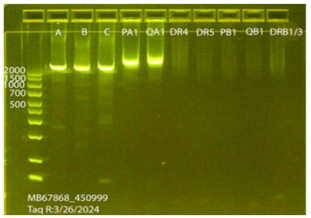

Figure 1. Lot #581844, received 3/26/2024, Taq Polymerase.

One patient for all 11 loci amplified. 50% of target loci failed to amplify. 24 patients, 11 loci, across 3 amplification plates are run on every NGS assay. These failures were not isolated to HLA-Class II and seemed sporadic between loci on the same assay as well as runs themselves. Class II amplification was impacted to a greater extent from the faulty Taq polymerase. This is likely due to the manufacture's primer set for Class II loci, which are designed to produced longer amplicon needed for extended read length during NGS analysis.

RFU values - Plate locus:

	1	2	3
A	6.39E+03	6.66E+03	6.47E+03
B	6.73E+03	7.05E+03	6.70E+03
C	6.43E+03	7.57E+03	7.27E+03
D	2.81E+03	5.44E+03	
E	7.30E+03	1.66E+02	
F	6.43E+03	5.95E+03	
G	2.05E+02	6.36E+03	
H	7.13E+03	7.23E+03	

Undiluted concentration (ng/µL) - Plate locus:

	1	2	3
A	142.1	148.2	143.8
B	149.6	156.9	149.1
C	143.1	168.7	161.9
D	61.8	120.8	-1.4
E	162.5	2.3	-1.4
F	143.1	132.3	-1.4
G	3.2	141.4	-1.4
H	158.8	161.0	-1.4

Figure 2. Lot# 581844, Taq polymerase screen.

Inventory was becoming critically low, and it was crucial the laboratory could identify acceptable vials of Taq polymerase. 19 different vials were used to amp the same patient DNA using the same DPB1 primer. With an expected value >115.0 ng/ml, 16 of 19 vials were acceptable. Due to the packaging of Taq polymerase by the third-party vendor, the percentage of acceptable vials out of a shipment of 62 was not possible to determine due to random selection of vials for the Taq screen.

The laboratory could not dedicate resources to test all 186 vials of Lot# 581844.

Investigative Results

The investigation consisted of multiple runs, using a combination of each component, analysis of our NGS workbooks and running agarose gels. The cause of the amplification failures proved to be the Taq polymerase provided with the NGS kits. The Taq vials, which are supplied by a third-party vendor, were all from the same manufacturing lot but only some were defective (Table 1).

Table 1

Receive Date	Number of Vials Delivered	Number of Vials used with Amp failures	Number of Vials used without failures
02/16/2024	62	0	34
03/26/2024	62	6	8
05/23/2024	62	5	26

In total, 186 vials of Taq Polymerase Lot# 581844 were received. Once ClinImmune's internal investigation was initiated, the shipments of Taq polymerase were quarantined and only used after additional screening was performed.

Conclusions

- On the surface, a problem with Taq polymerase is the obvious answer to failed amplifications. However, coming to this conclusion was difficult.
- During the set-up of our assay, the technologist uses 2 – 3 vials of Taq polymerase across three amplification plates. All individual vials of Taq polymerase, from the same shipment, also had the same lot number.
- After the Taq polymerase was quarantined, we tested each individual vial for quality of amplification and discovered only one in six vials resulted in a failed amplification.
- Because the issue was isolated to specific vials of Taq polymerase of the same lot, a technologist could have a perfect run, a partial amp or failure across all three plates.
- This variability made it hard to ascertain which reagent was causing the problem.
- Careful tracking of amplification issues allowed us to identify the culprit and was a good reminder that reagents from the same manufacturing lot can potentially be problematic.