Extracting gDNA from Clotted Specimens: Validating the “Crushed Clot”

Carly Carozza[1], Michael Ponisciak[1], Mary Carmelle Philogene[2], Noureddine Berka[1]

[1]Histocompatibility Laboratory Services, American Red Cross, Philadelphia, PA

[2]Histocompatibility and Immunogenetics Laboratory, Virginia Commonwealth University Medical Center, Richmond, VA

AIM

In efforts to avoid sample rejection, additional invasive procedures to patients, and delays in testing, our laboratory investigated extracting genomic DNA (gDNA) from clotted blood specimens. We assessed the quantity and quality of gDNA from clotted specimens and ensured lack of inhibition in any of our primary PCR-based assays. We also assessed concordance of testing results obtained from gDNA extracted from clotted blood specimens with results obtained by established acceptable sample sources.

METHODS

Twenty clotted blood specimens with previous HLA typing were selected for extraction and NGS typing.

Specimens were centrifuged at 3000g for 10 minutes and serum was discarded. PBS was added at a 1:10 ratio to the clot and the clot was repeatedly pierced with wooden applicators for approximately 5 minutes to break up large debris. An aliquot of the "crushed” clot/PBS solution was utilized for DNA extraction (Figure 1).

DNA extraction was performed on the Maxwell RSC using the Buffy Coat DNA Kit (200ul sample input), and the KingFisher Flex using the MagMAX Multi-sample Ultra 2.0 Kit (250ul sample input), with 100ul elution. DNA concentration and quality was measured on the NanoDrop and diluted as needed for downstream applications using DNA grade nuclease-free water.

All gDNA obtained from clotted specimens were typed by NGS (AllType). To ensure lack of inhibition, several gDNA were tested by additional PCR-based methods to establish concordance between results obtained and various previously reported results: SSOP (LABType), STR (GlobalFiler), SBT (SeCore), and NGS (AlloSeq Tx17).

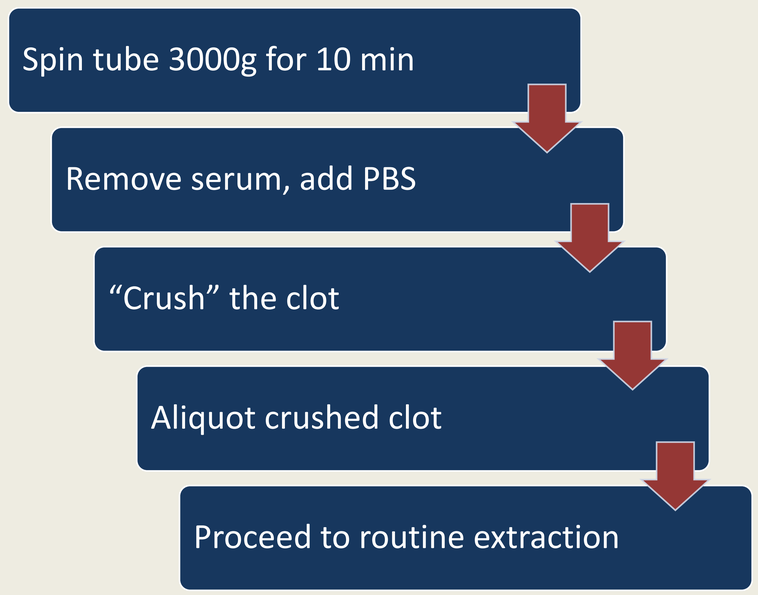

Figure 1. Workflow of clot isolation and manipulation to prepare for DNA extraction

RESULTS

DNA quantity and quality obtained from the clotted specimens were comparable between extraction kits (Table 1). Samples with low DNA concentration were cleaned using AMPure XP beads (Beckman Coulter) to meet criteria for downstream PCR-based applications. DNA yield averaged 67.3 ng/ul by Maxwell RSC with an average purity of 1.83 and 68.6 ng/ul by KingFisher Flex with average purity of 1.82. All 20 samples achieved concordance between previous typing and NGS typing obtained from gDNA from the clotted specimen. A selection of these results along with demonstration of lack of inhibition is shown in Table 2. Due to the testing requests for the initial typing of the specimens, not all samples had 11-loci typing originally performed and are designated as not tested (NT). Sample 14 was tested by all primary test methods in our laboratory.

		Maxwell RSC			KingFisher Flex		
Sample ID	Clot Source	Concentration (ng/ul)	A260/A280	A260/A230	Concentration (ng/ul)	A260/A280	A260/A230
1	Deceased Donor	20.7	1.82	1.32	30.3	1.88	1.79
2	Deceased Donor	22.2	1.86	1.40	29.2	1.74	1.41
3	Deceased Donor	40.3	1.87	1.63	58.3	1.88	2.16
4	Deceased Donor	42.4	1.91	1.82	90.3	1.91	2.23
5	Deceased Donor	14.9	1.90	0.96	12.4	1.71	1.40
6	Platelet Refractory	166.9	1.84	1.79	166.9	1.90	2.14
7	Platelet Refractory	28.2	1.75	0.55	14.8	1.62	1.41
8	Platelet Refractory	17.4	1.78	0.41	4.6	2.03	0.99
9	Platelet Refractory	187.0	1.86	1.99	290.8	1.92	2.10
10	Platelet Refractory	101.0	1.81	1.58	116.4	1.88	2.00
11	Platelet Refractory	80.7	1.81	1.31	78.2	1.88	2.08
12	Platelet Refractory	20.6	1.68	0.52	10.2	1.67	1.10
13	Platelet Refractory	40.9	1.86	0.98	26.3	1.73	1.67
14	Healthy Donor	91.7	1.92	1.37	82.4	1.80	1.92
15	HSCT Patient	57.8	1.83	1.24	37.7	1.77	1.77
16	HSCT Patient	81.5	1.81	1.37	62.2	1.89	1.96
17	HSCT Donor	110.5	1.83	1.82	101.5	1.90	2.07
18	Platelet Refractory	29.1	1.74	0.86	19.0	1.74	1.37
19	Platelet Refractory	66.2	1.84	0.68	52.0	1.78	1.77
20	Healthy Donor	126.5	1.86	1.43	88.5	1.80	1.88
Average		67.3	1.83	1.25	68.6	1.82	1.76

Table 1. Clotted blood extraction method comparison

CONCLUSIONS

Clotted blood specimens were proven to be an acceptable source of gDNA for testing. 100% typing concordance was observed between previous sources and clotted blood specimens and there were no inhibitory effects observed in any PCR-based methods assessed. This is a cost effective and accurate sample preparation method accessible to all laboratories without the need for additional extraction kits.

	Clot gDNA Genotype by Alltype NGS									Additional PCR-based methods											
ID	A*	B*	C*	DRB1*	DRB3/4/5	DQA1*	DQB1*	DPA1*	DPB1*	A*	B*	C*	DRB1*	DRB3/4/5	DQA1*	DQB1*	DPA1*	DPB1*	Method	Concordant (Y/N)	Inhibition (Y/N)
1	02:06	35:01	07:02	04:07	4*01:03	03:01	03:02G	01:03	03:01G	02:XX	35:XX	NT	04:XX	NT	NT	NT	01:XX	03:XX	SSOP	Y	N
	23:01	39:06	07:02	08:01G		04:01	04:02G	01:03	04:02G	23:XX	39:XX	NT	08:XX	NT	NT	NT	01:XX	04:XX			
2	02:01	07:02	02:02	11:01	3*02:02	01:02	03:01G	01:03	04:01G	02:XX	07:XX	NT	11:XX	NT	NT	NT	NT	NT	SSOP	Y	N
	24:02	40:02	07:02	15:01G	5*01:01G	05:05	06:02G	02:06	05:01G	24:XX	40:XX	NT	15:XX	NT	NT	NT	NT	NT			
3	01:01	07:02	06:02	04:01	4*01:03	02:01G	02:01G	01:03	04:01G	01:XX	07:XX	NT	04:XX	NT	NT	NT	NT	NT	SSOP	Y	N
	02:05	50:01	07:02	07:01	4*01:03	03:03	03:01G	01:03	20:01G	02:XX	50:XX	NT	07:XX	NT	NT	NT	NT	NT			
4	11:01	44:03	15:02	07:01	4*01:01G	02:01	02:01G	01:03	04:02G	11:XX	44:XX	NT	07:XX	NT	NT	NT	NT	NT	SSOP	Y	N
	29:02	51:01	16:01	07:01	4*01:03	02:01	02:01G	02:01	17:01G	29:XX	51:XX	NT	07:XX	NT	NT	NT	NT	NT			
5	01:01	08:01	04:01	04:01	4*01:03	03:01	03:01G	01:03	04:01G	01:XX	08:XX	NT	04:XX	NT	NT	NT	NT	NT	SSOP	Y	N
	11:01	35:01	07:01	04:04	4*01:03	03:03	03:02G	01:03	04:01G	11:XX	35:XX	NT	04:XX	NT	NT	NT	NT	NT			
14	01:01	08:01	04:01	03:01	3*01:01G	05:01	02:01G	01:03	04:01G	01:01:01	08:01:01	04:01:01	03:01:01G	3*01:01:02G	05:01:01	02:01:01	01:03:01	04:01:01	NGS (AlloSeq)	Y	N
	02:01	35:03	07:01	11:04	3*02:02G	05:05	03:01G	01:03	04:01G	02:01:01	35:03:01	07:01:01	11:04:01G	3*02:02:01G	05:05:01	03:01:01	01:03:01	04:01:01			
14	01:01	08:01	04:01	03:01	3*01:01G	05:01	02:01G	01:03	04:01G	01:01:01G	NT	NT	NT	NT	NT	NT	NT	NT	SBT	Y	N
	02:01	35:03	07:01	11:04	3*02:02G	05:05	03:01G	01:03	04:01G	02:01:01G	NT	NT	NT	NT	NT	NT	NT	NT			
14	STR Results: D3S1358-16, 18; vWA-18, 19; D16S539-9, 11; CSF1PO-10, 11; TPOX-9, 11; Yindel-2; Amel-X, Y; D8S1179-13, 13; D21S11-28, 28; D18S51-12, 19; DYS391-11; D2S441-11, 12; D19S433-15.2, 15.2; TH01-6, 9.3; FGA-24, 24; D22S1045-16, 17; D5S818-11, 11; D13S317-11, 13; D7S820-12, 12; SE33-28.2, 29.2; D10S1248-13, 14; D1S1656-15.3, 16; D12S391-20, 20; D2S1338-21, 24									Previous STR Results: D3S1358-16, 18; vWA-18, 19; D16S539-9, 11; CSF1PO-10, 11; TPOX-9, 11; Yindel-2; Amel-X, Y; D8S1179-13, 13; D21S11-28, 28; D18S51-12, 19; DYS391-11; D2S441-11, 12; D19S433-15.2, 15.2; TH01-6, 9.3; FGA-24, 24; D22S1045-16, 17; D5S818-11, 11; D13S317-11, 13; D7S820-12, 12; SE33-28.2, 29.2; D10S1248-13, 14; D1S1656-15.3, 16; D12S391-20, 20; D2S1338-21, 24									STR	Y	N

Table 2. Sample selection demonstrating typing concordance and lack of inhibition across multiple PCR-based methods; NT = Not Tested

CONTACT

Carly Carozza
American Red Cross
Histocompatibility Laboratory Services
700 Spring Garden Street
Philadelphia, PA, 19123
Carly.Carozza@redcross.org
215-451-4277