

Evaluation of RBC Hydration After Treatment with S-303 Pathogen Inactivation at Varying Hematocrits (HCT)

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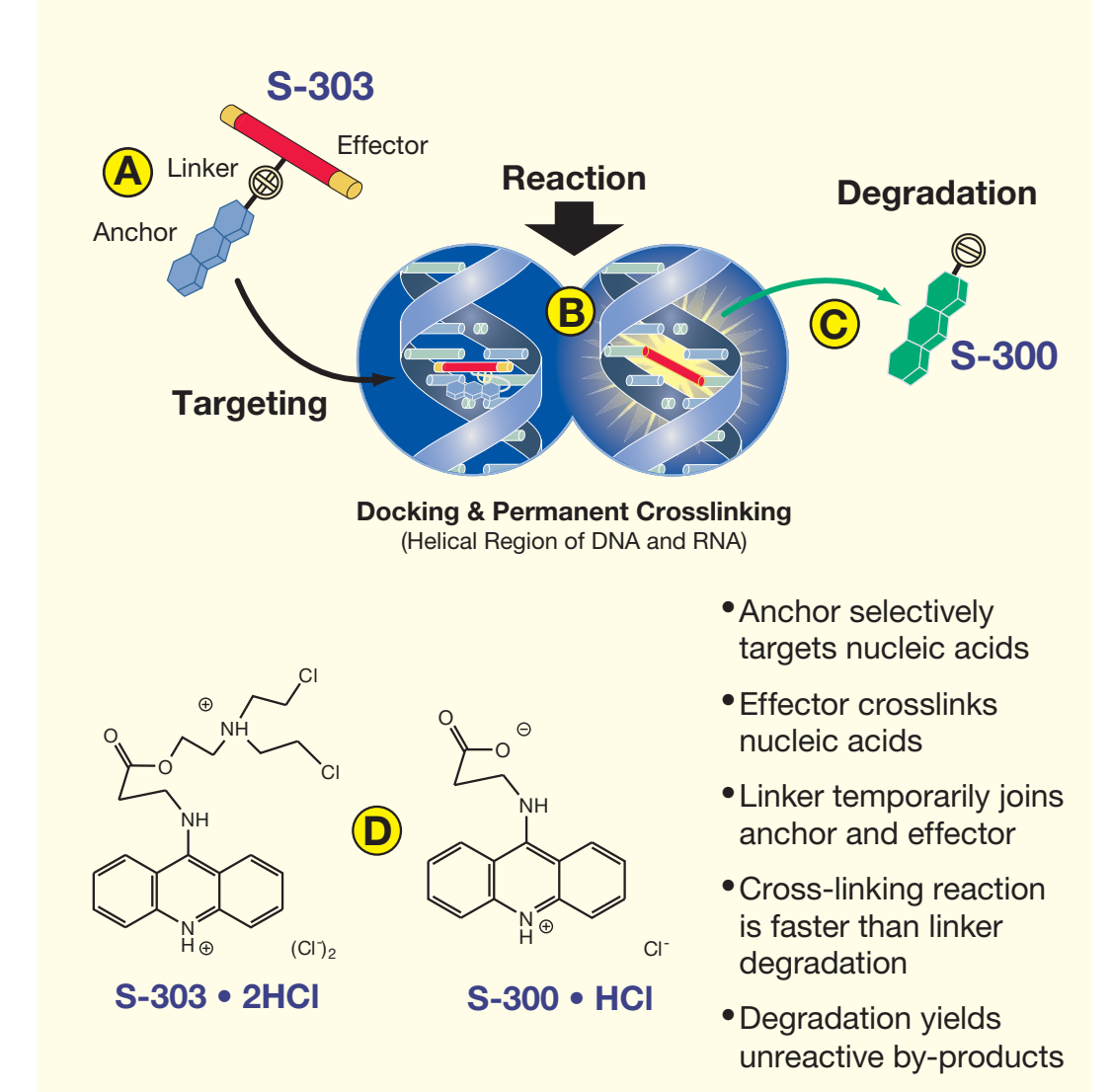


Background

The S-303 pathogen inactivation (PI) system for Red Blood Cells (RBC) uses the small molecule S-303 to form covalent cross links with nucleic acids of contaminating pathogens and prevent their replication. S-303 is a modular compound (Figure 1A-1C) designed to spontaneously decompose by hydrolysis to the non-reactive compound S-300 (Figure 1D). Quenching of unincorporated S-303 is achieved by including pH-adjusted glutathione (GSH), a natural antioxidant, in the treatment process.

Cerus has been evaluating the S-303 PI process by focusing on the effects of treatment on RBC cellular hydration. Since treated RBC may be stored for up to 6 weeks, we have been assessing the functional and physical characteristics of RBC treated with S-303 during storage under standard blood banking conditions. These studies have explored several treatment options for the S-303 treatment process that 1) preserves RBC in vitro properties predictive of viability, specifically cellular hydration, while maintaining robust pathogen inactivation activity and 2) can be readily integrated with current blood banking practices.

Figure 1: S-303 Treatment Process Mechanism of Action



Aim

To characterize S-303 treated RBC using osmotic fragility, mean corpuscular hemoglobin concentration (MCHC) and osmotic gradient ektacytometry (EKTA) as indicators of RBC hydration starting with RBCs at different levels of percent hematocrit and stored in SAGM for 6 weeks at 4°C.

Methods

S-303 Treatment Process

Red cell concentrates (RCC) were prepared from leukoreduced whole blood units from 450 mL collections. For comparison, whole blood units were pooled after leukoreduction and divided equally, into full units, prior to preparation of RCC. RCCs were treated at approximately 80%, 60%, or 40% HCT as described in Figure 2. Test units were treated with a GSH sodium salt and S-303 at

final concentrations of 20 mM and 0.2 mM respectively. Test units were incubated up to 20 hours at RT. After RT incubation, units were centrifuged and the supernatant was exchanged with 100 mL of SAGM prior to storage at 4°C. Control RBC units were prepared in SAGM and stored at 4°C. All units were evaluated over approximately 6 weeks of storage at 4°C. Two separate studies were performed in duplicate; averages for the duplicates are

represented in the following figures. In the first study SAGM (Control A) was compared to the 80% and 40% HCT process options while in the second study, the 60% HCT process was compared to SAGM (Control B).

Assays of RBC Hydration

Mean corpuscular hemoglobin concentration (MCHC) and spun hematocrit were measured manually. Osmotic

fragility (a measure of RBC surface area-to-volume ratio (S/V) and a traditional indicator of the hydration state of the cell) measures the degree of resistance of RBC to lysis as a result of a change in the cell volume. Increases and decreases in osmotic fragility are defined by shifts in the hemolysis curves and are typically expressed using median corpuscular fragility (MCF) which is the osmolarity at which the cell population exhibits 50% lysis (Figure 3). The

method used in this study is taken from Lew et al., 2003. In a separate study, Test units treated with the 40% HCT process and corresponding Controls (Day 36 post donation) were subjected to osmotic gradient EKTA to examine the effects of treatment on RBC membrane deformability. Samples for EKTA analysis were tested both with and without washing three times with 0.9% saline.

Figure 2: S-303 Treatment Process with Varying Hematocrits

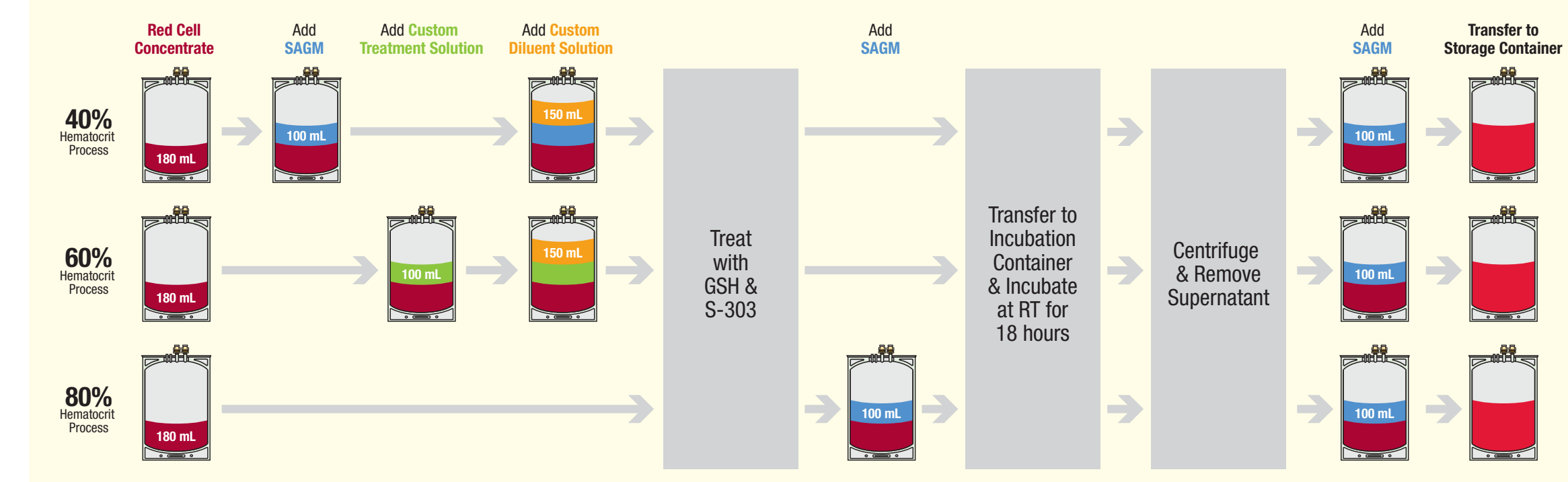
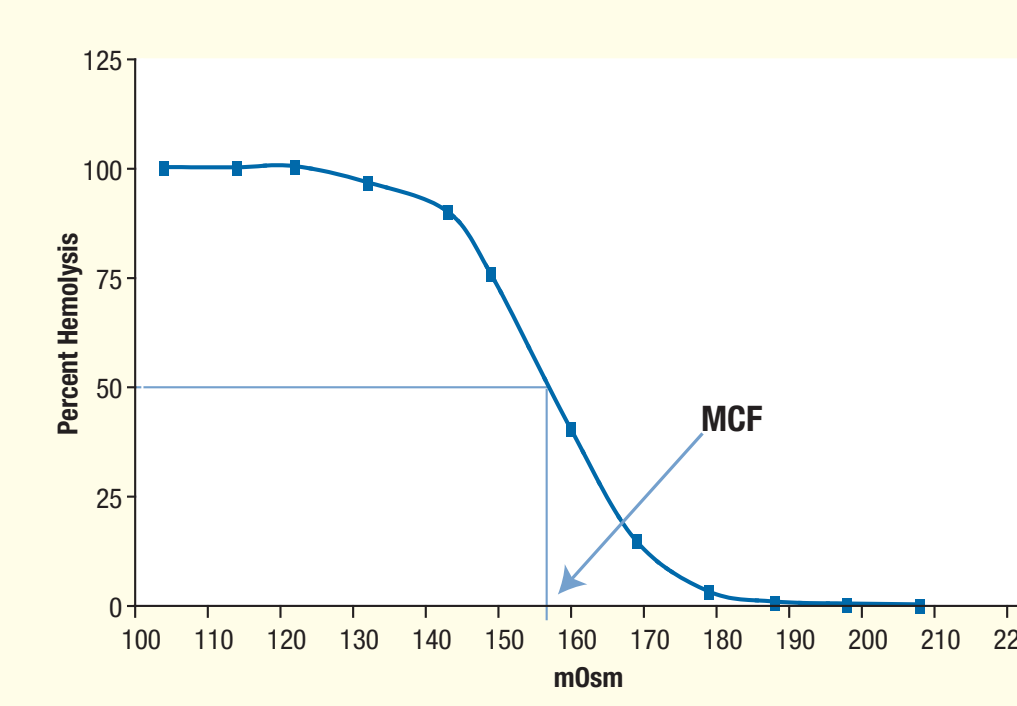


Figure 3: Graph demonstrating how MCF is measured



References

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Conclusion

Storage of S-303 treated RBC for up to 6 weeks did not significantly alter the RBC hydration.

- The MCHC of the Test units for all HCT treatment processes was not significantly different from Controls.
- Osmotic fragility and EKTA showed no significant differences between Control and Test, regardless of treatment process.

Results

Test and Control units had similar MCHC and spun HCT both 2 days after the PI process and throughout 6 weeks of storage at 4°C (Table 1 and Table 2). This indicated the hydration status of Test cells was comparable with untreated Control RBCs. Using more sensitive measures, the osmotic fragility profile of the 40% HCT treatment was similar to Control 2 days after treatment with only a subtle shift towards decreased fragility (Figure 4) and slightly decreased MCF at the end of storage (Table 3). The units treated with the 60% HCT Process showed a similar profile to that of the 40% HCT process with a slight left shift (Figure 5; Table 3). For units treated with the 80% HCT Process, the osmotic fragility curve shifted towards increased fragility compared to Control (Figure 4). However, after approximately 6 weeks of storage these units had MCF values similar to Control (Figure 4; Table 3).

RBC hydration was also assessed by osmotic gradient EKTA; a technique that uses a laser-diffraction viscometer to study changes in the water content and

membrane surface area of RBC with high sensitivity. Cytoplasmic viscosity is a major determinant of erythrocyte deformability; consequently, RBC dehydration has substantial rheological implications in that impaired deformability can cause a reduction in cell survival (Mohandas et al. 1979; Clark et al. 1983; Clark 1989; Da Costa et al. 2001). Using RBCs treated with the S-303 process at 40% HCT, deformability was assessed after 6 weeks of storage with osmotic gradient EKTA. The changes in membrane surface area and corresponding changes in red cell deformability is expressed as DI (deformability index) and DI_{max}. Comparison of Test cells from the 40% HCT treatment with untreated Controls at Day 36 shows the DI_{max} of both to be below normal range (0.54 ± .06), but washing the cells with physiological saline and allowing them to equilibrate before analysis raised both Test and Control to within the normal range for fresh whole blood (Test = 0.55 ± .03; Control = 0.58 ± .02; p = 0.05) showing that the storage induced reduction in DI_{max} is reversible in both cases (Figure 6).

Figure 4: Osmotic Fragility of 40% and 80% HCT Treatment Processes vs. SAGM Control After 2 and 38 Days of Storage

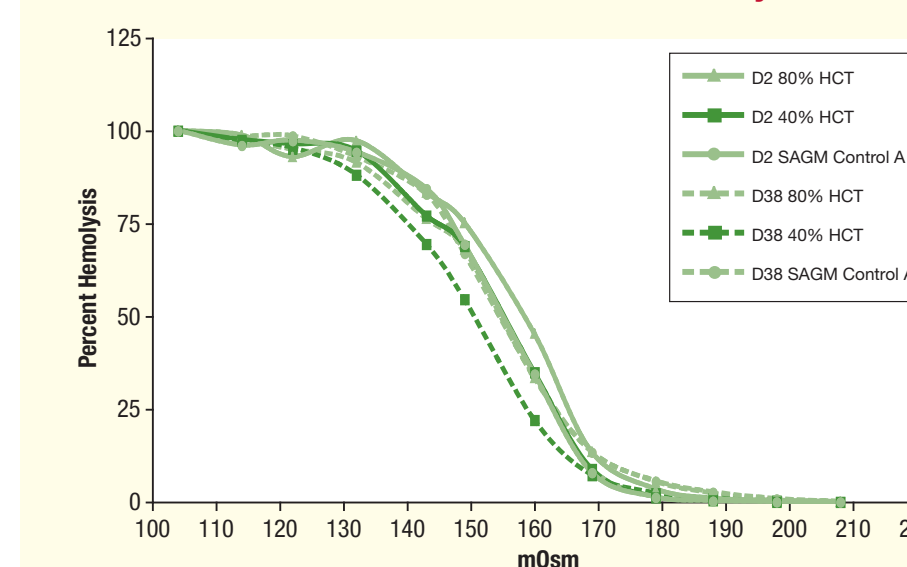


Table 1: MCHC for 40%, 60%, and 80% HCT Treatment Processes vs. SAGM Control Over 6 Weeks Storage

Days Post Donation	MCHC (g/dL)				
	80% HCT	40% HCT	SAGM Control A	60% HCT	SAGM Control B
2	33	32	33	33	34
7-9	34	32	31	32	32
20-22	35	33	33	32	33
36-38	34	33	32	34	33
42-44	34	32	33	33	32

Figure 5: Osmotic Fragility of 60% HCT Treatment Process vs. SAGM Control After 2 and 36 Days of Storage

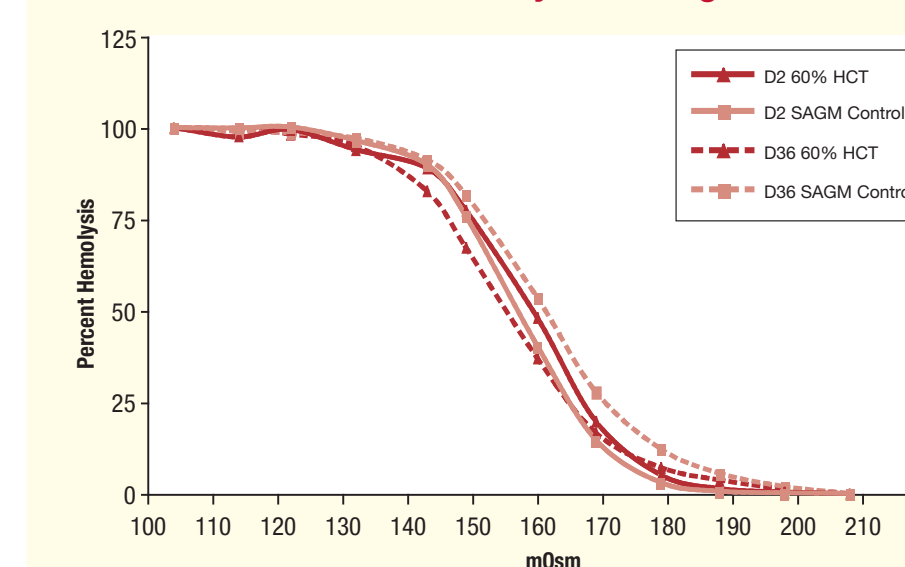


Table 2: Spun Hematocrit for 40%, 60%, and 80% HCT Treatment Processes vs. SAGM Control Over 6 Weeks Storage

Days Post Donation	Spun Hematocrit (%)				
	80% HCT	40% HCT	SAGM Control A	60% HCT	SAGM Control B
2	63	60	60	59	60
7-9	61	60	61	61	63
20-22	61	60	60	59	61
36-38	62	59	60	58	62
42-44	61	59	59	58	61

Figure 6: DI_{max} Measurements for 40% HCT Treatment Process vs. SAGM Control After 36 Days of Storage

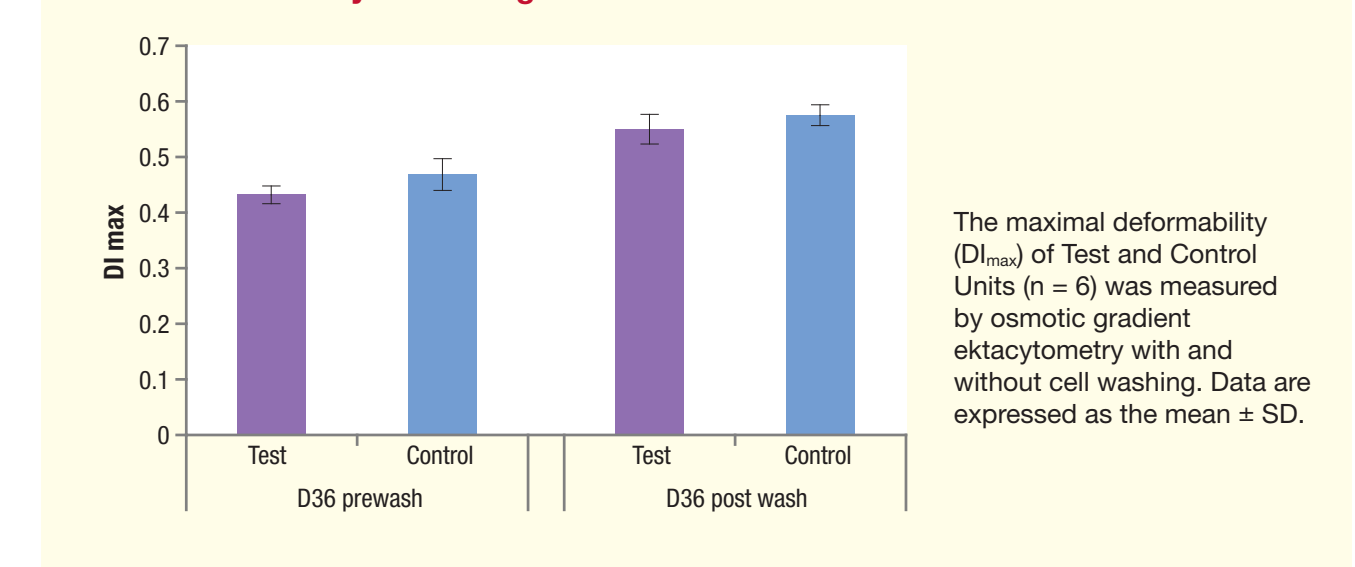


Table 3: MCF (Mean values) for 40%, 60%, and 80% HCT Treatment Processes vs. SAGM Control Over 6 Weeks Storage

Days Post Donation	MCF (mOsm)				
	80% HCT	40% HCT	SAGM Control A	60% HCT	SAGM Control B
2	159	155	156	155	154
7-9	156	153	155	153	153
20-22	155	151	155	149	153
36-38	154	149	155	151	158
42-44	156	150	158	151	158