

Performance Qualification of the ACP 215: Blood Bank of Hawaii's Validation Experience



Laurie A. Davis, MLS(ASCP)SBB^{CM}
Technical Director, Blood Bank of
Hawaii
Honolulu, Hawaii



Objectives

- Develop an understanding of FDA-approved applications for the ACP 215
- Describe key parameters measured in validating the ACP 215 protocols
- Discuss challenges we encountered during validation and strategies employed to address them
- Identify potential off-label applications for the ACP 215

Background

The Haemonetics ACP 215 is an automated, closed-system cell processor for red blood cells-

- Washing
- Automated glycerolization
- Deglycerolization

With the upcoming sunseting of the COBE 2991, the ACP will be the only licensed instrument available in the US to perform these processes

Limitations of the ACP 215

Protocols only currently FDA-approved for use with whole-blood-derived red blood cells

Currently no protocols available in the US for freeze/deglyc of apheresis rbc, washing of platelets, making of red cell stroma, processing cell therapy products, etc.

Washing

Per the Haemonetics manual, washing is only approved for whole-blood derived red blood cells

- Stored in
 - CPDA-1 for up to 21 days
 - CPD/AS-1 for up to 28 days
- Leukoreduced prior to storage
- Does not allow for apheresis red blood cells
- 24 hour expiration date

Verification criteria

AABB Standard 5.7.4.6- WASHED RED BLOOD CELLS

“Washed Red Blood Cells shall be prepared by a method known to ensure that the red cells are washed with a volume of compatible solution that will remove almost all of the plasma”

We opted to also measure % recovery as part of our process validation

Verification results

12 units were washed on ACP 215

- % recovery was calculated- acceptable result $\geq 80\%$
 - 100% of units recovered $\geq 80\%$ of original red cell mass
- Verification of adequate protein removal using protein sticks- acceptable result $\leq 1+$
 - 100% of units tested $\leq 1+$ protein

Glycerolization

Glycerolization with the ACP 215 automates an often manual process

- Currently approved for whole-blood-derived RBC stored in
 - CPD/AS-1, CP2D/AS-3 (LR) or CPD/AS-5 (LR or non-LR)
- Closed system glycerolization allows for products to have a 14-day expiration

Verification criteria

The Haemonetics manual states:

- Unit should be glycerolized and frozen within 6 days of collection (this must happen to obtain a 14-day outdate)
- RBC must be transferred to 800-1000 mL storage bag, qualified for freezing
- Hematocrit of initial RBC should be ~75% (64-82%) prior to initiating glycerolization- remove additive/plasma
- Excess glycerol must be removed prior to freezing to achieve a post-thaw hematocrit of ~60 % (44-70%)

Difficulties with glycerolization

We noted we had problems with the bag that Haemonetics recommended-

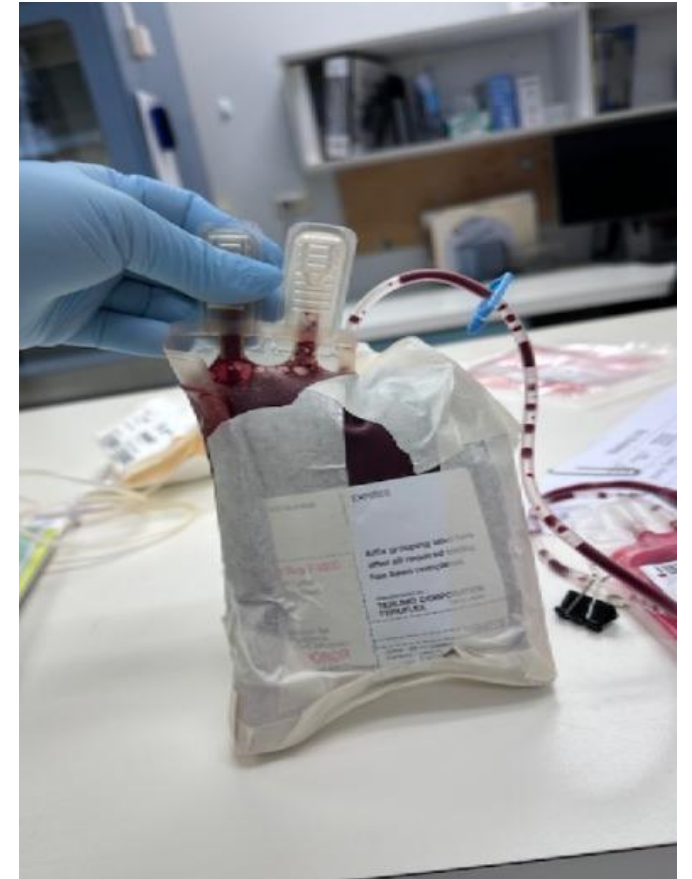
- Difficulties with bag fitting in our centrifuges
- We chose to use a Terumo 1000 mL bag for freezing, no issue with centrifugation or breakage of these bags



Difficulties with glycerolization

We found that, to spin the units for expressing additional glycerol, we got a better separation by taping the bags

- They fit better in the centrifuge cups
- Easier to express additional glycerol



Glycerolization validation

The manual states the product, after removal of additional glycerol, should have a hematocrit of 44-70% upon thaw

- We chose to validate this pre-freeze so we could standardize (as much as possible) how much glycerol is removed
- Lesson learned: mix the glycerolized unit really well prior to centrifugation for additional glycerol removal

Results

12 units were processed using the glycerolization protocol

- Evaluating the hematocrit pre-freeze allowed us to experiment with how much glycerol to express to hit the target listed in the manual- a hematocrit measured after thaw did not show a significant difference from pre-freeze value

Units were utilized for validation of the deglycerolization process

Deglycerolization

The deglycerolization protocol on the ACP 215 is used for deglycerolization of:

- Red blood cells, derived from whole blood collections, non-LR, stored in CPDA-1, glycerolized using the ACP 215 (14 day exp. possible)
- Red blood cells stored in CPD/AS-1, CP2D/AS-3 (LR) or CPD/AS-5 (LR or non-LR), glycerolized using the ACP 215 (14 day exp. possible)
- Red blood cells collected in other anticoagulant/additive solution, glycerolized using the ACP 215 (24 hour exp.)
- Red blood cells, non-LR glycerolized by manual Valeri method (24 hour exp.)

Deglycerolized product expiration

14-day outdate

- Products glycerolized on ACP 215 in closed system, frozen within 6 days of collection

24-hour outdate

- Products glycerolized on ACP in closed system, frozen > 6 days
- Products glycerolized on ACP in open system
- Products manually glycerolized and deglycerolized on ACP (Valeri)

Verification criteria

AABB Standard 5.7.4.5- DEGLYCEROLIZED RED BLOOD CELLS

“Deglycerolized Red Blood Cells shall be prepared by a method known to ensure adequate removal of cryoprotective agents, result in minimal free hemoglobin in the supernatant solution, and yield a mean recovery of $\geq 80\%$ of the preglycerolization red cells following the deglycerolization process”

Verification criteria

Per the Haemonetics manual:

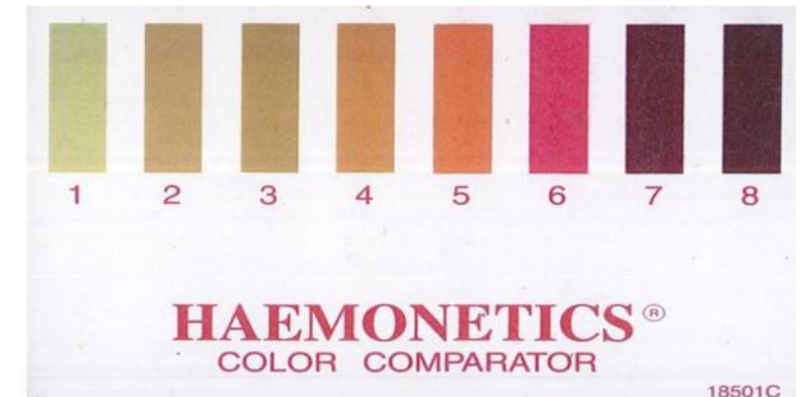
The deglycerolization protocol on the ACP 215 produces products:

- Minimum average RBC recovery of 80%
- Maximum level of hemolysis is 1% after deglycerolization and 14 days of storage at 1-6°C
- Maximum osmolality of 400 mOsm/kg H₂O

BBH deglycerolization verification

Used the 12 units frozen during the glycerolization verification

- Units were kept frozen at -80C for a minimum of 14 days prior to deglycerolization
- Units were thawed and deglycerolized per manufacturer instructions
- Evaluation criteria:
 - % Recovery ($> 80\%$)
 - Free Hgb < 150 mg% (ACP printout)
 - Supernatant Hgb level ≤ 4 (Color comparator chart)
 - Osmolality ≤ 400 mOsm/kg H₂O



Results

- All products had Osmolality result of < 400 mOsm/Kg H₂O
- All products recovered Free Hgb% of $< 50\text{mg}\%$
- All products' supernatant hgb level < 2 compared to hemolysis chart
- All products % recovery $> 80\%$

Conclusions

- We found process verification for washing of RBC to be fairly straightforward and did not encounter any issues of note
- The glycerolization process required some “trial and error”
- Validating the intermediate process with a pre-glyc and pre-freeze HCT ensured a smoother verification for the deglycerolization process- all products acceptable
- Overall, the staff is happy with the use of the ACP for these protocols

Where do we go from here?

Exploring off-label use of the ACP-

- Deglycerolization of Meryman units- come see our abstract poster! (P-BC-73)
- Glycerolization/Deglycerolization of apheresis red blood cells
- Possibilities for washing of platelets?

Questions?

