Practical Aspects of Pre-Storage Leukocyte Reduction in a Blood Establishment Including Use of Pooled Samples for Enumeration of Residual Leukocytes

G. F. Leparc, M.D.
Chief Medical Officer
Florida Blood Services
St. Petersburg, Florida

Timothy P. Malone, MT(ASCP)SBB
Technical Director
Florida Blood Services
St. Petersburg, Florida

FLORIDA BLOOD SERVICES

Keys to Successful Quality Control of Blood Components

Q.C. processes should be designed to provide assurance of safety, identity, purity and potency of blood components while:

- minimizing loss of transfusable components
- being performed under feasible logistics
- adding reasonable cost

FLORIDA BLOOD SERVICES

Pre-Storage Leukocyte Reduced Components

- Platelets Pheresis by Automated Collection
  - Baxter AMICUS
  - Gambro TRIMA
- RBC by Filtration
  - AS-1, AS-5, AS-3
- RBC by Automated Collections
  - Baxter ALYX

FLORIDA BLOOD SERVICES

Platelets, Pheresis Collections at FBS

- Average of 1,200 collections of Platelets by apheresis method per month
- Approximately 2,000 transfusion doses per month
- Service area demand is slightly above 3,000 transfusion doses a month
- Whole blood-derived platelets make up the difference

FLORIDA BLOOD SERVICES

Platelets, Pheresis Collections at FBS

- Collection sites: 11
- Total number of instruments placed: 39
- Collection instrument types (2):
  - Baxter Amicus (30)
  - Gambro Trima (9)

FLORIDA BLOOD SERVICES

Quality Control of Platelets, Pheresis

- Daily:
  - Performed on 100% of units collected.
    - CBC, including PLT and WBC count
    - Bacterial culture for aerobic bacteria and fungi
    - Calculation of total platelet contents and unit volume (in mL)

FLORIDA BLOOD SERVICES

Quality Control of Platelets, Pheresis

- Identifying Components for Monthly QC:
  - Performed Counts on a minimum of 4 units per month
  - Perform pH (> 6.2)
    - For each component type: Single, Double, and Triple dose collection
    - For each site
    - For each collection instrument

    (rotating instruments at each site)

FLORIDA BLOOD SERVICES

Monthly QC Scorecard

		PHN	PHD	PHT
FBM	4059			
	4479			
	4480			
	Any			
NDM	2 x 3403	--	--	--
	2 x 4494	--	--	--
CLM	4680			
	4681			
	4682			
	5252			
Largo	4507			
	4060			
	4670			
	Any			
PCM	2 x 5089	--	--	--
	2 x 5090	--	--	--
KB	2 x 5242	--	--	--
	2 x 5243	--	--	--
BLA	2 x 8819	--	--	--
	2 x 8846	--	--	--

		PHN	PHD	PHT
Brandon	5196			
	5197			
	5198			
	5199			
Alternate months	5269			
	5270			
TPM	4481			
	4495			
	5149			
	5150			
Alternate months	5152			
WES	4058			
	4643			
	5151			
	Any			
MAN	8850			
	8866			
ODD MONTHS	8867			
	8870			
EVEN MONTHS	8872			
	8874			
	8876			

END OF STORAGE QC

NEED 4 (EACH) PER MONTH

AMICUS		TRIMA	

FLORIDA BLOOD SERVICES

Quality Control of Platelets, Pheresis

- Monthly QC – Performed at End of Storage or at "Time of Issue"
  - ✓Platelet Count **(must be at least > 3 x 10^{11} in 100 % of components)**
  - ✓WBC Count **(must be < 5 x 10^{6} WBC in 100 % of units)**
  - ✓pH **(must be > 6.2 in 100% of components tested)**
  - ✓For each component code (single, double, triple) as per Division of Hematology, CBER.

FLORIDA BLOOD SERVICES

Quality Control of Platelets, Pheresis

- Units identified for Monthly QC (based on Daily QC) are held until Day 4 of storage for testing, and released if QC passed
- Effect on component outdate rate:
  - 34% of units held for QC (avg. 72/mo.) expire on shelf

FLORIDA BLOOD SERVICES

Quality Control of Platelets, Pheresis

- Monthly QC Testing schedule
  - ✓ 4 Singles = 4 **components**
  - ✓ 4 Doubles = 8 **components**
  - ✓ 4 Triples = 12 **components**

  = 24 **components for QC, per site**

  X 11 sites

  = 264 Components in QC per month, held to "End of Storage" or "Time of Issue"

FLORIDA BLOOD SERVICES

Current Monthly QC costs*

- Platelet count: $900
- Residual WBC count by flow cytometry: $7,900
- Component pH: $25
- Component outdate: $35,250

Total monthly cost: $44,000

*** Excluding bacterial detection QC**

FLORIDA BLOOD SERVICES

QC of RBCs Pre-Storage Leukocyte Reduced

- RBC by Filtration Baxter Sepacell R2000
  - average 2,500/mo
  - 1% of Production (perform 30/mo)
    - Residual WBC by Flow
    - % Recovery (vol:vol)

- RBC by Automated Collections Baxter ALYX
  - Average 400 donations / mo = 800 u
    - Hgb conc – min 25 donations = 50 u/site
    - Residual WBC by Flow - 4 u QC per mo / collection site

FLORIDA BLOOD SERVICES

Questions to consider

- Do the current QC requirements offer a significant **additional** assurance on the quality of components prepared?
- Given the cost in testing and lost components, as well as the lack of statistical significance of QC requirements in the current Guideline, will we be able to use a more rational QC model?

FLORIDA BLOOD SERVICES

Beyond QC

- Each location where apheresis products are collected requires a separate license
- Licensing of each site requires submission of platelets components to FDA for each component type (Single, double, or triple dose)
- Collection systems (i.e. instrumentation and storage bags) are licensed for the preparation and storage of components with defined expiration time

FLORIDA BLOOD SERVICES

Proficiency Testing

- CAP Survey TRC – Transfusion Related Cell Counts
  - Methodology
    - Manual Counts using Nageotte Hemacytometer
    - Automated Flow Cytometry
  - Dichotomy of Test Methods
    - 125 Labs performing Nageotte
    - 49 Labs performing Flow

FLORIDA BLOOD SERVICES

Proficiency Testing

- Consolidated Data Summary of the most recent TRC Challenge With Expected Results of ~ 6 M Residual WBCs.
  - Nageotte Testing Labs:

    55% LR vs 45 % non-LR
  - Flow Testing Labs

    99% LR vs 1% non-LR

FLORIDA BLOOD SERVICES

Quality Control of Leukoreduced Blood Components

Is Universal Q. C. testing in a pooled configuration feasible?

FLORIDA BLOOD SERVICES

Precedents for Pooled Testing Exist

- Nucleic acid testing (NAT)
  - HIV
  - HCV
  - WNV
- Bacterial contamination detection in Whole Blood-Derived Platelets

FLORIDA BLOOD SERVICES

FBS Sampling Data for Residual WBCs in Apheresis Platelets

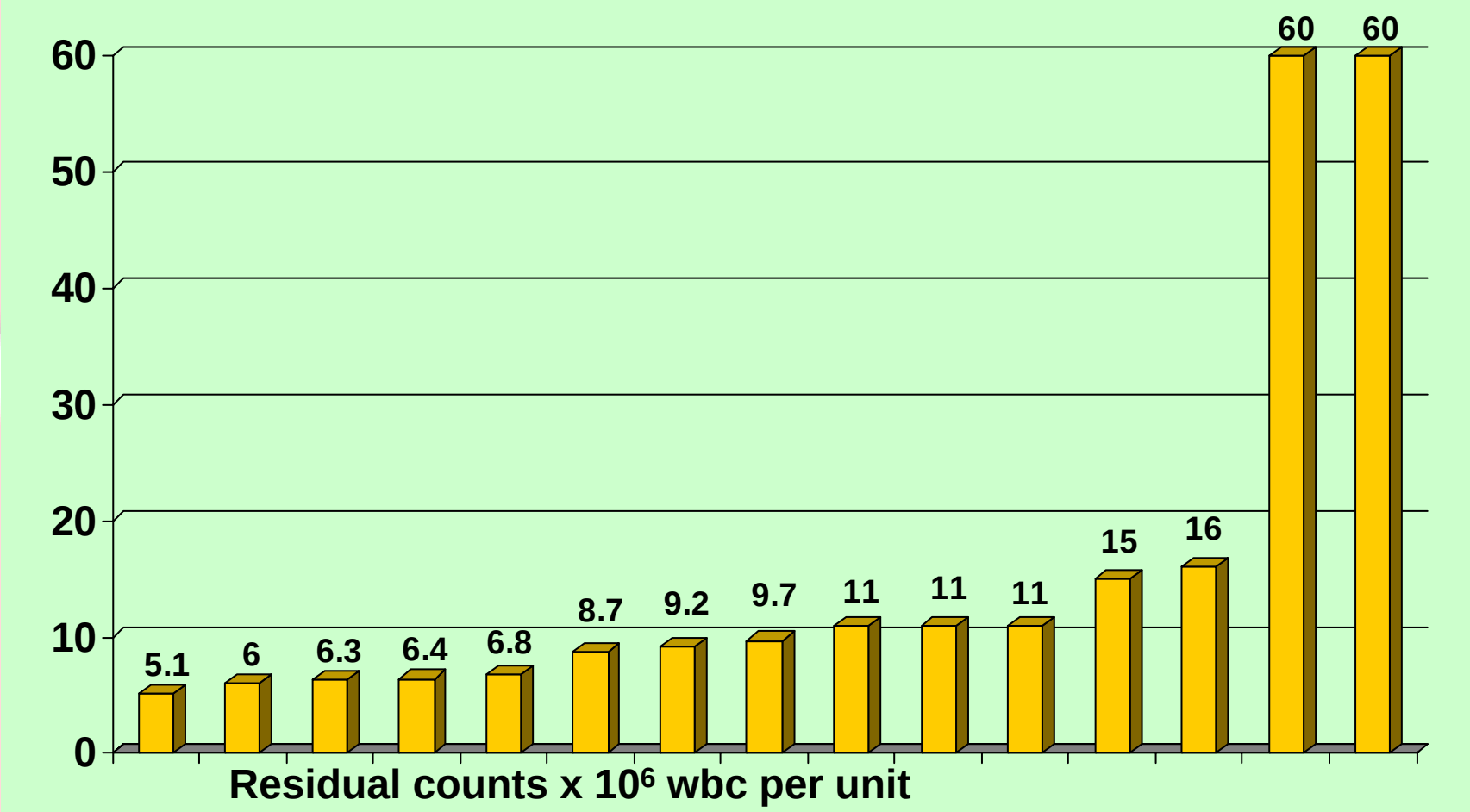
- Total units tested: 1,555
  - 1% of Components Produced

- No. units that failed* Q. C.:
  - n = 15 (1.0% Failure Rate)

* Failure due to residual wbc count >5 x 10^6/unit

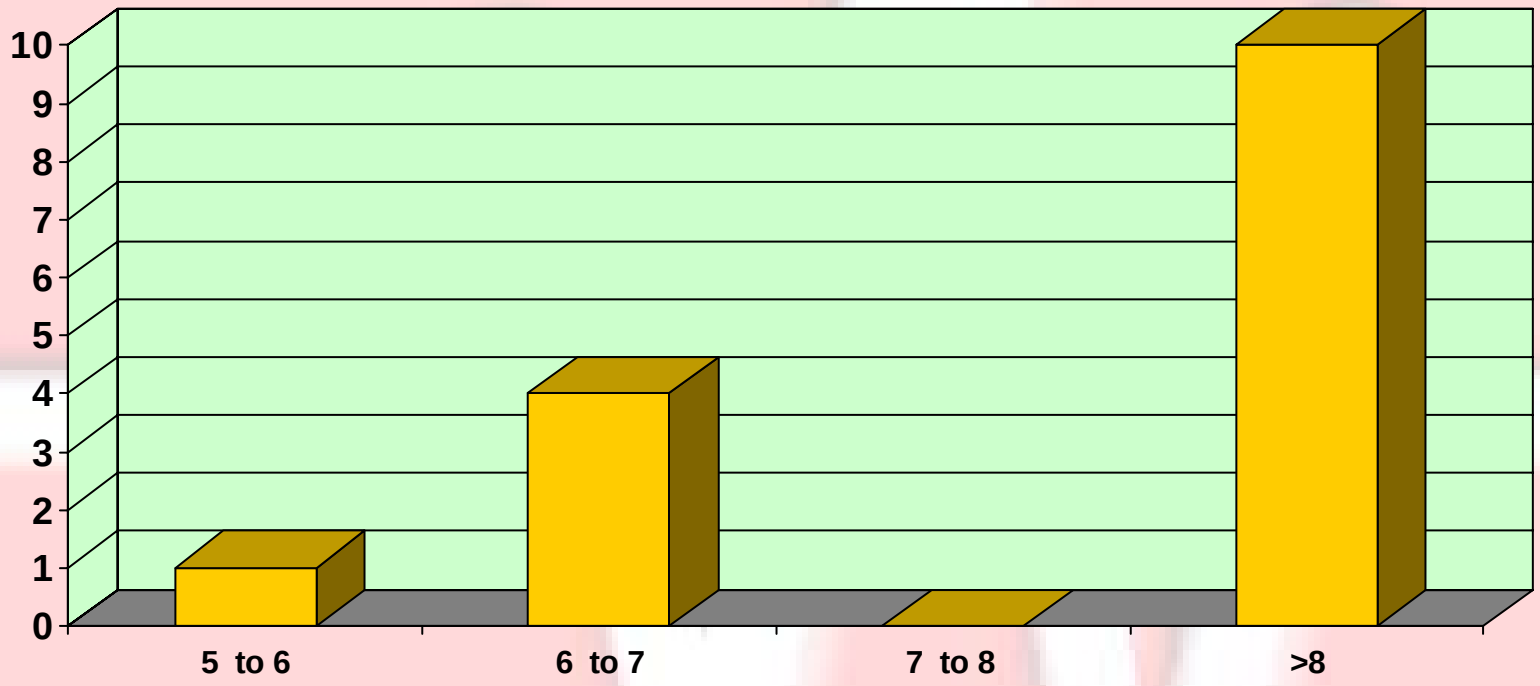
FLORIDA BLOOD SERVICES

Distribution of Residual WBC counts on failed units



FLORIDA BLOOD SERVICES

Distribution of Residual WBC counts on failed units



Number of units with failed WBC counts within intervals (x 10^6)

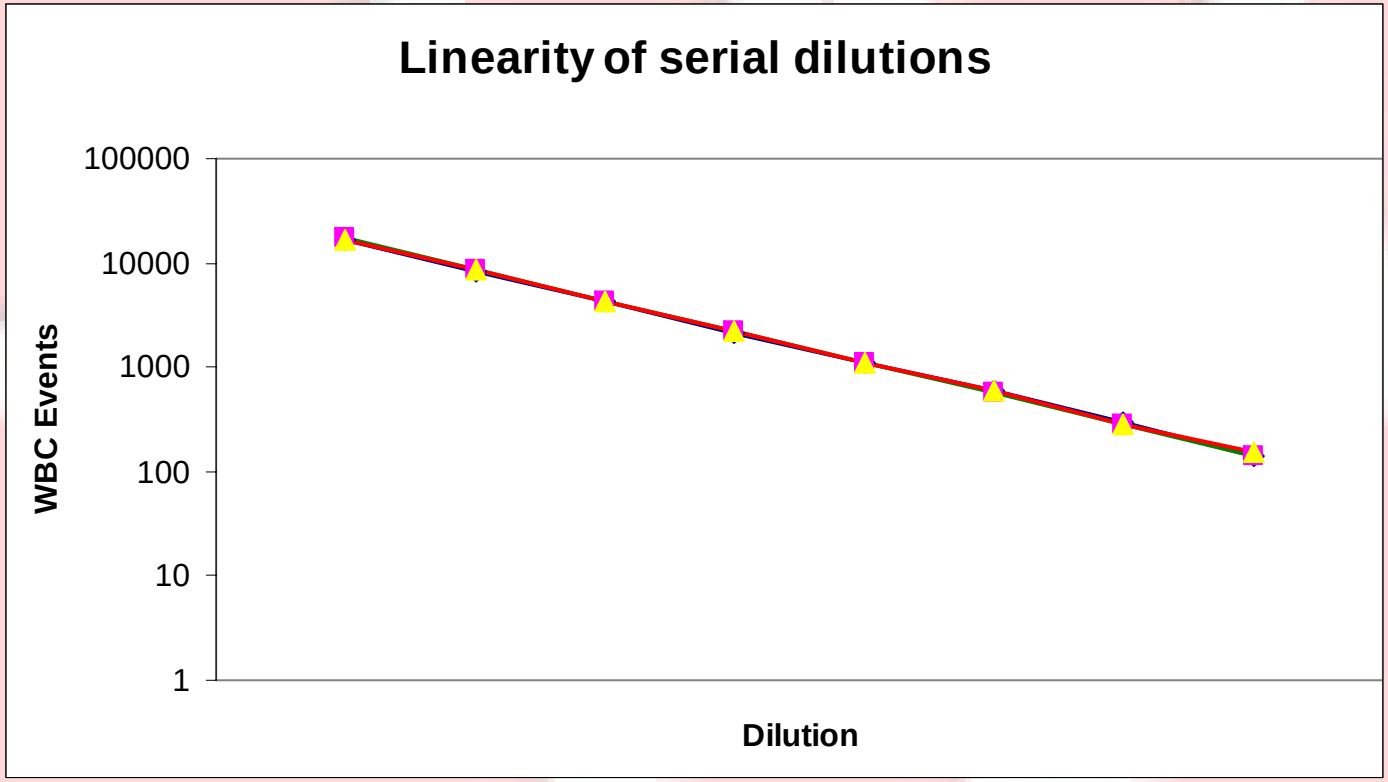
FLORIDA BLOOD SERVICES

Pool Testing Procedure Highlights

- Individual pool member volume: 1 mL
- Total members in pool: 10
- Sample volume tested by flow cytometry: 100 µL
- Instrument: Becton Dickinson FACSCalibur Flow Cytometry System
- Reagent: LeukoCount Test Kit

FLORIDA BLOOD SERVICES

Linearity of dilutions



Linearity results on 5 replicates

FLORIDA BLOOD SERVICES

Cut-off calculation methodology

Must take account of Residual WBC contribution by successfully leukodepleted units:

R_{WBC} c/o = $RBD_{WBC\ events}$ + WBC c/o $_{events}$

RBD_{WBC} = $\bar{x}$ WBC $_{events}$ on LP-units X 9

FLORIDA BLOOD SERVICES

Cut-off calculation methodology

- Greater sensitivity is achieved if

  $\bar{x}$ – 2 SD WBC_{events} is used to calculate RBD_{WBC} but the rate of pool "failure" climbs from 1% to up to 5%

FLORIDA BLOOD SERVICES

Cut-off calculation methodology

- Parameters are specific and must be calculated for every type of leukodepleted components:
  - Apheresis PLT leukodepleted at collection
  - Automated RBC collection
  - In-line filtration
  - Etc.

FLORIDA BLOOD SERVICES

Conclusions

- Preliminary data show that:
  - Universal Q.C. testing of leukodepleted blood components is feasible
  - Significant additional testing needs to be performed to determine
    - Sensitivity and specificity rates
    - Effect of pooling of ABO incompatible samples
    - Performance in comparison with statistical sampling

FLORIDA BLOOD SERVICES