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Department of Pathology
Peoria, IL 61636

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REAGENT QUALITY CONTROL

Principle

Good laboratory practice requires reagents be tested on the day of use to ensure that the products are reactive and specific. Any deviation from the expected result warrants investigation prior to use. Reagents are tested when first placed in service. When new lot numbers of reagents are received, a note should be left either on the board or on the QC log so the technologist performing the QC the next day can QC the new lot number. The exception to this rule is when you QC a lot number immediately and place it into service**.** New lot numbers are recorded in the Master Reagent Lot Tracking List, along with a date placed into service. Daily-use reagents are QC'd by first shift every day. QC for other reagents is performed when needed for use in patient testing. All QC performed must be logged on the appropriate sheet.

Clinical Significance

In order to ensure that the patient results are accurate you must be able to assure the reagents and equipment you are using will perform as expected. This must be done prior to reporting any patient results.

Reagents

See Master Reagent Lot Tracking List for reagent information
Daily Use Reagents, to be performed Daily.
Anti A, Anti B, Anti D, Anti AB, Anti-IgG, C3b (Polyspecific), Anti-IgG
A1/B Cells, Gel Screen cells, IgG Gel cards, Coombs Check Cells

Instrumentation/Equipment

Incubators – Gel and Tube
Centrifuges – Gel and Cell Washer / Immufuges
Impact Pipette / BioHit Pipette/ Finnpipette

Procedure: Inspect all reagents being used for evidence of contamination or deterioration (i.e. marked turbidity of the antiserum, hemolysis of the red cells). Check the IgG card for signs of discoloration, bubbles, crystals, drying (a liquid layer should appear on top of the Gel in each microtube), or seals which appear damaged or opened.

All reactions should be evaluated for possible shifts in reaction strength.

Reporting Results:

1. Pull one reagent vial of each lot number to be used and ensure all lot numbers are recorded in the Master Reagent Lot Tracking List.
   -Move vials to student rack and use new lot if necessary.

2. Record all results for daily antisera/reagent use on the Monthly Blood Bank QC form (Daily Reagent Quality Control).
   **Day of Use Reagents are recorded on the Monthly Blood Bank QC form (Day of Use Reagent Quality Control)*

3. For reagents that require visual checks ensure that the lot number of the reagent being used is the lot number that currently in use. Verify lot number and expiration and report interpretation as pass.

4. Any deviations from the expected results should be brought to the attention of the team leader. This discrepancy must be resolved prior to using the equipment or reagents for patient testing.

Daily Use Antisera and Reagent Red Cells:

1. Label test tubes 1-10.
2. To tubes 1 and 2 add 1 drop of polyspecific antihuman globulin.
3. To tube 3 and 4 add 1 drop of Anti-A.
4. To tubes 5 and 6 add 1 drop of Anti-B.
5. To tubes 7 and 8 add I drop of Anti-A,B.
6. To tubes 9 and 10 (add 1 drop of Anti-D.
7. To tubes 3 and 6 add 1 drop of A1 cells.
8. To tubes 4 and 5 add 1 drop of B cells.
9. Add 1 drop of Combscell-E IgG coated red blood cells to tube 1.
10. Add 1 drop of CorQC reagent cells (group AB, D positive) to tubes, 7 and 9.
11. Add 1 drop of Internal QC (ARC group O negative cells) to tubes 2, 8 and 10.
12. Mix and centrifuge all tubes for 15 seconds at 3400 RPM. Examine macroscopically for agglutination. Grade and record reactions and interpretations on the QC log sheet.
13. Discard all tubes except tube 10.
14. Incubate tube 10 for 15 minutes at 37 degrees. After incubation, wash 3 times with normal saline. Add 2 drops of Anti-IgG, mix well and centrifuge for 15 seconds at 3400 RPM. Examine macroscopically for agglutination.
15. Grade and record the reaction, confirming the validity of the negative test tube 10 with 1 drop of Combscell-E IgG coated RBCs. Centrifuge and record results on the QC log sheet.
16. CorQC is a visual check only.
17. Discard tubes when finished

For the **Gel QC**: **QCGSC &QCcard**

1. Label 4 wells of the MTS Gel Card I and IIfor positive and negative reactions.
2. Remove the foil seal from the 4 wells to be used.
3. Using the Impact / Biohit pipette or the Finn pipette: Add 50 microliters of 0.8% cell suspensions of the Selectogen Gel Screening Cells into the appropriately labeled wells.
4. Add 25 microliters of corQC reagent antiserum into the positive reaction wells.
5. Add 25 microliters of saline into each of the negative reaction wells
6. Place the card in the Gel 37degree Incubator for 15 minutes. (Not to exceed 40 minutes)
7. After incubation, place the card in the Gel Centrifuge and spin for 10 minutes at 900 RPM.
8. Read the front and back of each microtube macroscopically and record the reactions.

QC for Diluent: (**QCDIL**) *Day of Use Reagent

1. Prime the MTS diluent dispenser so there is no evidence of bubbles.
2. Dispense 1ml of diluent into a properly labeled tube.
3. Add 10ul of donor packed red blood cells. Mix.
   *Alternatively, convert tube cells as needed.
4. Label 2 wells on a MTS Gel Card as Positive and Negative.
5. Remove the foil seal from the 2 wells and pipette 50ul of the donor unit cell suspension into both of the wells.
6. Add 25 microliter of corQC reagent antiserum to the positive well.
7. Add 25 microliter of saline to the negative well.
8. Place the card in the Gel 37degree Incubator for 15 minutes. (Not to exceed 40 minutes)
9. After incubation, place the card in the Gel Centrifuge and spin for 10 minutes at 900 RPM.
10. Read the front and back of each microtube macroscopically and record the reactions.

QC for Tube screen:

1. Label tubes #1-4.
2. Add 1 drop of Biotestcell reagent cells 1 to tubes 1 and 2.
3. Add 1 drop of Biotestcell reagent cells 2 to tubes 3 and 4.
4. Add 1 drop of corQC reagent antiserum to tubes 1 and 3 and 2 drops of saline to tubes 2 and 4. Mix
5. Centrifuge for 15 seconds at 3400 RPM. Examine macroscopically for agglutination and record results.
6. Add 2 drops of Albumin/OAES.

7. Mix and incubate at 37 degrees for 15 minutes for albumin or 10 minutes for OAES.
8. Centrifuge for 15 seconds at 3400 RPM. Examine macroscopically for agglutination and record results.
9. Place tubes in the cell-washer and wash 3 times with saline. Add 2 drops of Anti-IgG antiserum to each tube. Mix and centrifuge. Examine macroscopically for agglutination.
10. Record the reactions and interpretations.

QC for Compliment Check Cells:

1. Add 1 drop of Anti-C3b,C3d anti-human globulin to a properly labeled tube.
2. Add 1 drop of compliment control cells. Mix and let sit for 5 minutes.
3. Centrifuge for 15 seconds at 3400 RPM. Examine macroscopically for agglutination.
4. Record the reaction and interpretation

For subgroups of A:

1. Label two tubes as Positive and Negative.
2. Add one drop of Anti-A1 Lectin to each tube.
3. Add one drop of A1 red cells to Positive control tube.
4. Add one drop of A2 red cells to Negative control tube.
5. Mix and centrifuge for 15 seconds at 3400 rpm.
6. Re-suspend the packed cells by gentle agitation and examine for agglutination.
7. Record result.

Visual checks that require the lot number of reagent is checked to insure the correct lot number is being used for testing.

- Elution Kit II, Immucor
- ARC reagent cells
- CorQC reagents
- Fetal Hemoglobin Stain, Sure-Tech
- Saline

Procedural Notes/Problem-Solving Tips

1. A discrepancy in the AB and Rh antiserum.

a. May be due to a loss of reactivity. To determine if this is the case, repeat testing using a different vial of the same lot number. If this doesn't resolve the discrepancy repeat testing using a different lot number of antiserum.

b. May be due to under-centrifugation or over-centrifugation. Repeat testing taking care to observe centrifugation times indicated by last centrifuge calibration. (This can be found on the side of each centrifuge) If discrepancies are still noted, recalibrate the centrifuge.

2. A discrepancy with A1 or B Cells

a. May be due to loss of antigenicity. Repeat testing using different A1 or B Cells of the same lot number. If a new lot number is not available, or is discrepancy is still seen, repeat testing using a different source of A1 or B Cells. To enhance reactivity you may also incubate the A1 and B cells at room temperature for 15 to 30 minutes.

b. May be due to under-centrifugation or over-centrifugation. See steps to follow under #1 in resolving discrepancies in the AB and Rh antiserum.

3. A discrepancy involving tubes that are incubated.
-May be due to improper incubation. Repeat testing using a different 37C incubator. If results are acceptable, check original incubator for fluctuations in temperature. (Hot/Cold spots). Mark those wells in the incubator that should not be used for testing, or take the incubator out of service.

4. A discrepancy with the Screening Cells.
-May be due to loss of antigenicity of the Screening Cell(s). Repeat testing using a different vial of the Screening Cell. There will be times when we have access to another lot number of Screening cells, repeat testing with these cells if using a new vial of the same lot number does not resolve the discrepancy. If the discrepancy is still not resolved repeat testing using panel cells or cells known to possess antigen corresponding to IgG antibody specificity. (Anti-D and Anti-c). If the discrepancy still exists see how to resolve discrepancies due to Anti AHG reagent.

5. A discrepancy seen in tubes containing Anti-human serum (IgG).

a. May be due to improper washing or neutralization of the serum.
b. If automated washing was used – Check the saline source and lines to ensure proper flow. Check washer on multiple cycles to ensure proper fill of all tubes. Correct any problems encountered and repeat testing.
c. If manual washing was used, repeat testing taking care to ensure all cell buttons are completely re-suspended after each wash and decanting of saline is complete after last wash.
d. If your reactions are weaker than those normally seen, (or not reacting at all) check the Coombs serum by testing the original vial and a second vial of the same lot number as follow: Add one drop of Coombs control cells (CC's) to two drops of Anti-

AHG. Centrifuge and read. Compare the two sets of results. If the reactions with the two vials differ – with the original vial used having a lesser strength of reactivity the probable cause is neutralization of the vial. Repeat testing using a new vial of anti–AHG. If the reactions are essentially the same with both vials, the probable cause is loss of reactivity of the entire lot of anti-AHG. Repeat testing using a different lot of Anti-AHG.

6. A discrepancy seen with Coombs Control Cells.
   -May be due to loss of reactivity of the cells. Prepare your own IgG sensitized cells and repeat test using these cells for control phase.

7. If any antiserum or cells are found to be inadequate to use, they should be removed from the racks and returned to the company. Any reagent returned to the vendor or taken out of service for ineffectiveness should be recorded on the Reagent Deviations Log.

8. When testing kits with multiple reagents the reagents in each kit will not be used in conjunction with another lot number of the same kit. If one of the reagents in the kit runs out, or fails to perform as expected, the whole kit will be discarded if another kit with the same lot number is not available. The new kit will be placed in use, and QC will be performed at the time of use of this new kit.

9. All kits will be run in tandem with positive and negative controls from the current kit, when first received and prior to first use for patient testing.

References

Gamma QC Test Systems
Ortho Reagent Inserts
Immucor/Gamma Reagent Inserts
MMCI Blood Bank QC Manual / Mary McCallister

Author: Jane Harrington MLT. 09-03-2010

Medical Director Approval: Elizabeth Bauer-Marsh, MD

REVISION HISTORY (began tracking 2012)			
Rev	**Description of Change**	**Author**	**Effective Date**
1	Removed old reagents and added BIO-RAD reagents.	Kathy Turpin	05/29/14
0.2	Added negative controls to the QC procedures for Racks 1,2 and 4	Kathy Turpin	08/22/14
3	Significant revision. Removal of all LIS specific reference. Added Master Reagent Lot list reference. Updated procedure to include negative controls for gel screen. Revised Daily QC procedure for Anti-A/B to QC against A1/B Cells and vice versa.	Vincent Strow	4/11/16

Reviewed

Reviewed by	Date	Coordinator/Manager	Date	Medical Director	Date
K. Maher	1/16/12		1/16/12	Elizabeth A. Bauer-Lau MD	1/16/12
		Kathy L. Turpin	8/23/14	Elizabeth A. Bauer-Lau MD	8/29/14
V. Strow	4/11/16	Kathy L. Turpin	7/19/16	Elizabeth A. Bauer-Lau MD	7/21/16