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Introduction

George Washington serology study includes CMV IgG Avidity and Anti-HCV.

Infections with Cytomegalovirus (CMV), a member of the herpesvirus family, are common in men and are usually mild and asymptomatic. However, in pregnant women, newborns, and immunocompromised individuals CMV infections may pose a significant medical risk. CMV infection remains difficult to diagnose on symptoms alone since a high percentage of infections remains asymptomatic. In utero infection may result in sequelae of varying degrees including mental retardation, chorioretinitis, hearing loss and neurologic problems. Since the risk of in utero virus transmission and CMV related damage of the fetus is markedly increased during primary infection, reliable recognition of primary CMV infection is of high importance for pregnant women. Although presence of anti-CMV IgG reduces the likelihood of CMV related complications, it does not assure complete protection from disease. The functional binding affinity or avidity of IgG antibodies increases progressively over time after immunization, also known as maturation of the humoral immune response. High percentage of low avidity IgG antibodies may indicate a primary infection whereas high percentage of high avidity IgG antibodies may indicate a recurrent infection.

The ARCHITECT CMV IgG Avidity assay is a qualitative method of the chemiluminescent microparticle immunoassay (CMIA) for the determination of the avidity of IgG antibodies to Cytomegalovirus in human serum and plasma. It is used as an aid in the differentiation between primary and non-primary infection. If primary infection needs to be excluded, CMV IgG reactive samples should be tested for CMV IgM and CMV IgG Avidity. A positive CMV IgM result in combination with a low avidity result is a strong indicator for a primary CMV infection within the last 4 months.

HCV is a bloodborne virus. The presence of anti-HCV indicates that an individual may have been infected with HCV, may harbor infectious HCV, and/or may be capable of transmitting HCV infection.

The ARCHITECT Anti-HCV assay is a qualitative method of the chemiluminescent microparticle immunoassay (CMIA) for the detection of antibody to hepatitis C virus in human serum and plasma.

Specimen Collection, Preparation and Storage

Specimen type and Storage

- Specimen collected (5mL for adult and 1mL for neonates) in serum separator tube potassium EDTA tubed may be tested on Architect . Other specimen type refers to each assay package insert. Specimens are stored at -20^{o}C after testing.

Specimen preparation

- Follow the tube manufacture's processing instructions for serum and plasma collection tubes. Gravity separation is not sufficient for specimen preparation.
- Previously frozen specimens must be thaw thoroughly.
- Mix thawed specimens by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.
- To ensure consistency in results, specimens must be transferred to a centrifuge tube and centrifuged at ≥10,000 RCF (Relative Centrifugal Force) for 10 minutes before testing if
  - They contain fibrin, red blood cells, or other particulate matter,
  - They require repeat testing, or
  - They were frozen and thawed

  Transfer clarified specimen to a sample cup or secondary tube for testing
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or secondary tube. Care must be taken to transfer only the clarified specimen without the lipemic material.

Materials and Equipment

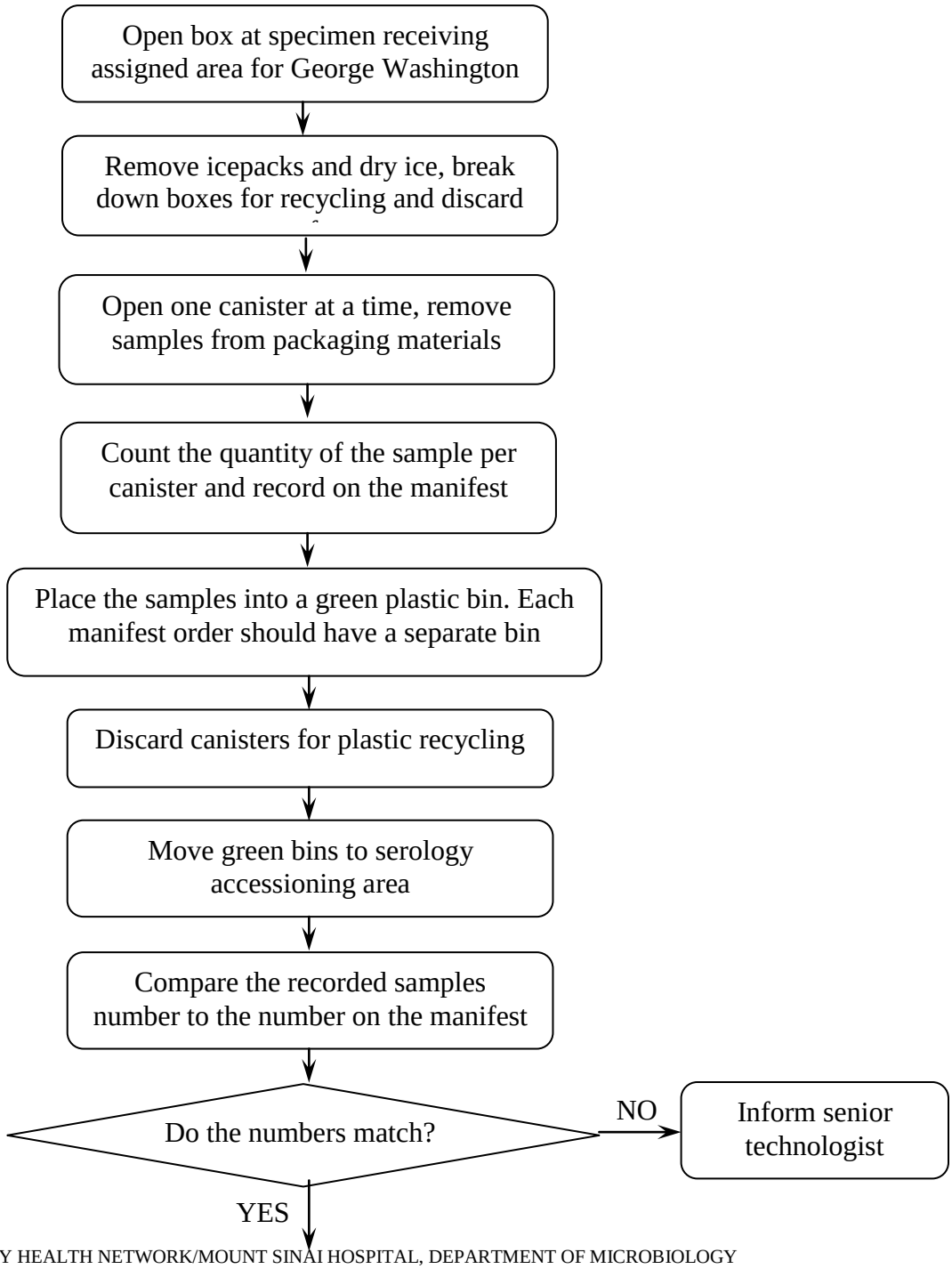
- ARCHITECT i System
- ARCHITECT i System Assay CD-ROM
- ARCHITECT i reaction vessels
- ARCHITECT i sample cups
- ARCHITECT i septum
- ARCHITECT i replacement caps
- Pipettes or pipette tips (optional)

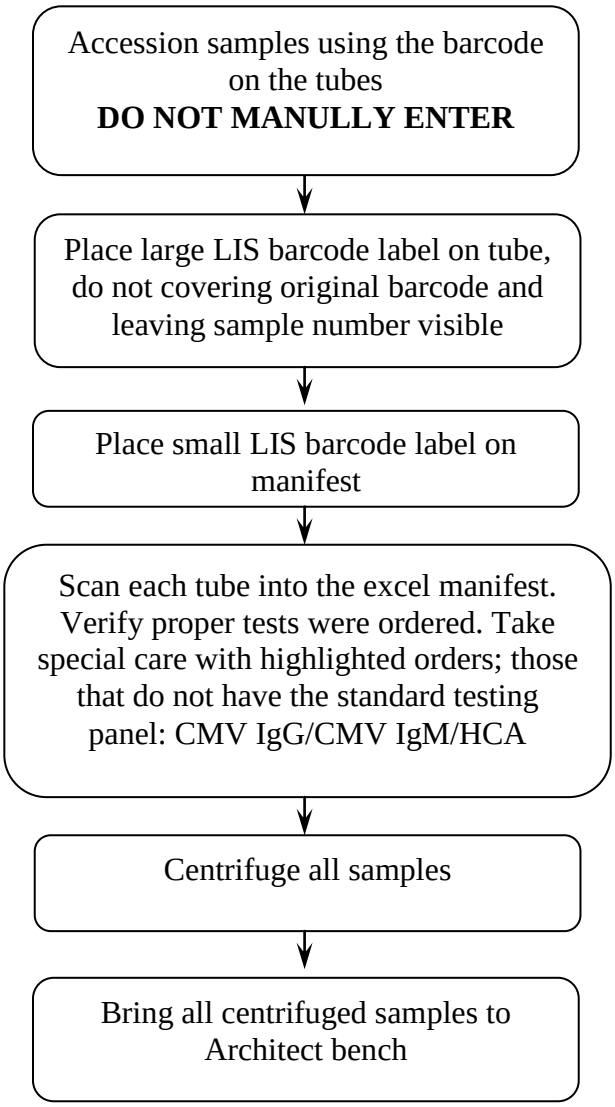
For information on materials required for maintenance procedures, refer to the Architect Manual.

- Abbott REAGENT KIT, CONTROL KIT, CALIBRATOR KIT : Must be carefully followed the instruction in each assay package insert. Stored at 4 ^{o}C.

- Abbot CONCENTRATED WASH BUFFER: Must be diluted prior to use. Contains 1.5M phosphate buffered saline with antimicrobial agents. Stored at room temperature.
- Abbott PRE-TRIGGER SOLUTION: Contains 1.32 %(w/v) hydrogen peroxide. Once opened, placed on board the system no longer than 28 days, then discards. Stored at 4 ^{o}C.
- Abbott TRIGGER SOLUTION: Contains 0.35N sodium hydroxide. Once opened, placed on board the systems no longer than 28days, then discard. Stored at room temperature.
- Abbott PROBE CONDITIONING SOLUTION: Contains recalcified human plasma; has infection risk. Preservatives: Antimicrobial Agent and ProClin 300. Stored at 4 ^{o}C.
- Ortho REAGENT KIT, CONTROL KIT: Must be carefully followed the instruction in package insert. Stored at 4 ^{o}C.

Specimen Management Procedure




Accession samples using the barcode on the tubes
DO NOT MANULLY ENTER
Place large LIS barcode label on tube, do not covering original barcode and leaving sample number visible
Place small LIS barcode label on manifest
Scan each tube into the excel manifest. Verify proper tests were ordered. Take special care with highlighted orders; those that do not have the standard testing panel: CMV IgG/CMV IgM/HCA
Centrifuge all samples
Bring all centrifuged samples to Architect bench

Testing Procedure

Prior to running specimens ensure:

- Maintenance on the architect was compelted: Architect SOP
- Ensure Architect Anti-HCV, CMV-IgG R , CMV-IgM, and CMV Avidity reagents on the system.
- Make sure all reagents have been calibrated and controls run. Refer toArchitect procedure.

1. Load George Washington specimen onto architect system
2. Once all tests are finished, the '**Result**' field will be flashing.
   - Print: '**Result lis Report**'
3. Touch '**Reruns**' from snapshot screen and delete all CMV-IgG reruns
4. Place all negative specimens in the aliquot rack. These specimens will be stored at -70 ^{o}C for 6 months
5. Aliquot any specimen with the value of Anti-HCV is less than 5.00 S/CO and store them in the positive CMV avidity + box (Freezer MIFTW beside the dark room).

6. **For Anti-HCV** ≥5.00S/CO, repeat Anti-HCV in duplicate on Architect:
   1) Both results <5.00S/CO , Anti-HCV is Negative, make an aliquot and put in positive CMV avidity + box (Freezer MIFTW beside the dark room)
   2) One result<5.00S/CO and one result ≥5.00S/CO, Anti-HCV is Positive, place an aliquot in the Evolis rack (MIRT8)
   3) Both results≥5.00S/CO, Anti-HCV is Positive, place an aliquot in the Evolis rack (Fridge MIRT8)
7. Refer Evolis HCV SOP to run Anti-HCV for George Washington

8. **For CMV IgM≥1.00 Index & CMV IgG Reactive**:
   - Order CMV Avidity **control**(Control L= Level 1, Control H= Level 2):
     1) Touch '**Order**', Touch '**Control order**'
     2) Scan in Carrier #. Type in position #
     3) Touch box with bar besi '**Control**', highlight CMV Avidity
     4) Make sure Level 1 is selected
     5) In Assay panel, touch **CMVAvidity**
     6) Touch '**Add order**'
     7) Touch box with bar besi '**Control**', highlight CMV Avidity
     8) Select '**Level 2**'
     9) In the Assay panel, touch '**CMVAvidity'**
     10) Touch '**Add order'**

11) Touch '**Exit'** to go back snapshot screen

- Order CMV Avidity for **patient's** specimen:
  - Touch '**Order'**, Touch '**Patient order'**
  - Scan in Carrier #. Type in position #
    1. At SID field, scan in specimen order#
    2. In panel field, touch **CMV G>AV** (CMV IgG R and zz-3009 are selected in Assay panel)
    3. Touch '**Sample details'**, type in Last name and First name
    4. Touch '**Done',** Touch '**Add order',** Touch '**Exit'**
  - Load CMV Avidity controls and patient's specimen
  - When CMV Avidity controls complete, print out the control list and release results
  - Check patient specimen order status
    1. Touch '**Order'**
    2. Touch '**Order Status'**
    3. Touch '**C/P'**
    4. Check the specimen order status

Proceed the following steps ONLY when both **CMV IgG R** and **zz-3009** complete

! DO NOT RELEASE ANY RESULT YET

9. Check CMV IgG R result
   a) If CMV IgG R≥250
      i. Reload patient's specimen onto Architect
      ii. Once CMV IgG R completes with an end-point value, Reload specimen onto Architect to get final CMV avidity result
   b) If CMV IgG R<250,
      i. Reload specimen onto Architect to get final CMV avidity result
10. Check patient's CMV Avidity result
   a) If CMV Avidity ≥50%
      i. Print and release related results: **CMVAvidity, CMVvi2, CMVAvi1, zz_3009, CMV IgG R**

b) If CMV Avidity<50%
   i) Print and release all related results: **CMVAvidity, CMVvi2, CMVAvi1, zz_3009 , CMV IgG R**
   ii) CMV Avidity and CMV IgM must be repeated.
   iii) Order CMV Avidity **AND** order CMV IgM at same time
   iv)Load patient specimen onto Architect
   v) Check patient specimen order status
   vi)Look for the specimen order status

Proceed the following steps ONLY when both CMV IgG R and zz-3009 complete
! DO NOT RELEASE ANY RESULT YET

11. Check CMV IgG R result
    a) If CMV IgG R≥250
       i. Reload patient's specimen onto Architect
       ii. Once CMV IgG R complete, which has end-point value, Reload specimen onto Architect to get final CMV avidity result
    b) If CMV IgG R<250
       i. Reload specimen onto Architect to get the final CMV avidity result

12. Check the 2nd CMV avidity result
    a. If CMV avidity result<50% (Matches the 1st avidity result)
       - Pritn and release related results: **CMVAvidity, CMVvi2, CMVAvi1, zz_3009, CMV IgG R, CMV IgM**
    b. If CMV avidity result ≥50% (Does not match the 1st avidity result)
       - Print but **DO NOT RELEASE** related results: CMVAvidity, CMVvi2, CMVAvi1, zz_3009 , CMV IgG R, CMV IgM
       - Repeat one more CMV Avidity test to get 2/3 results

13. Aliquot any CMV avidity positive specimen and store in CMV Avidity+ box (Freezer MIFTW)

CMV Avidity Trouble Shooting

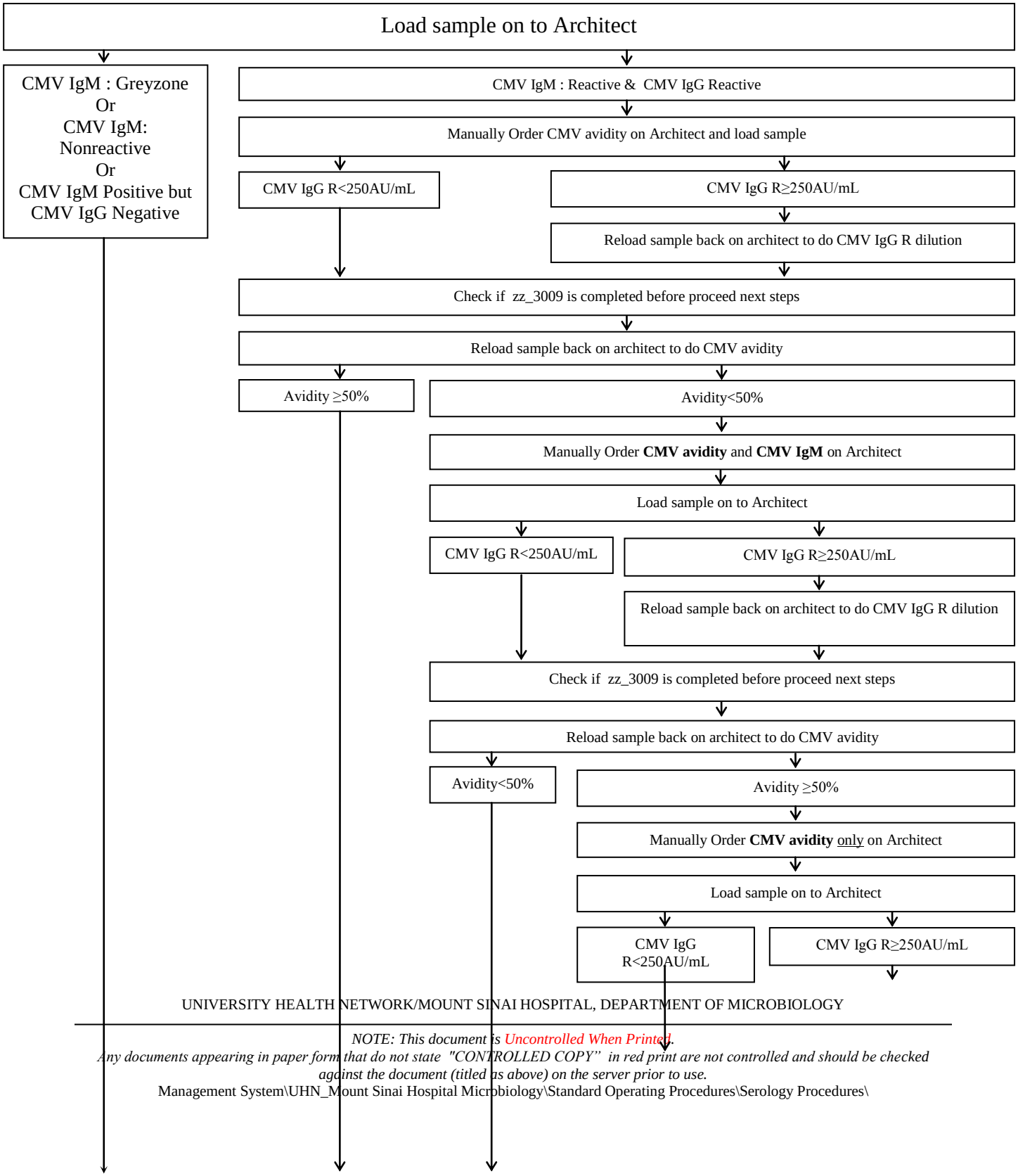
Unable to calculate CMV avidity due to high value of CMV IgG

- Perform 1:50 dilution on the specimen:

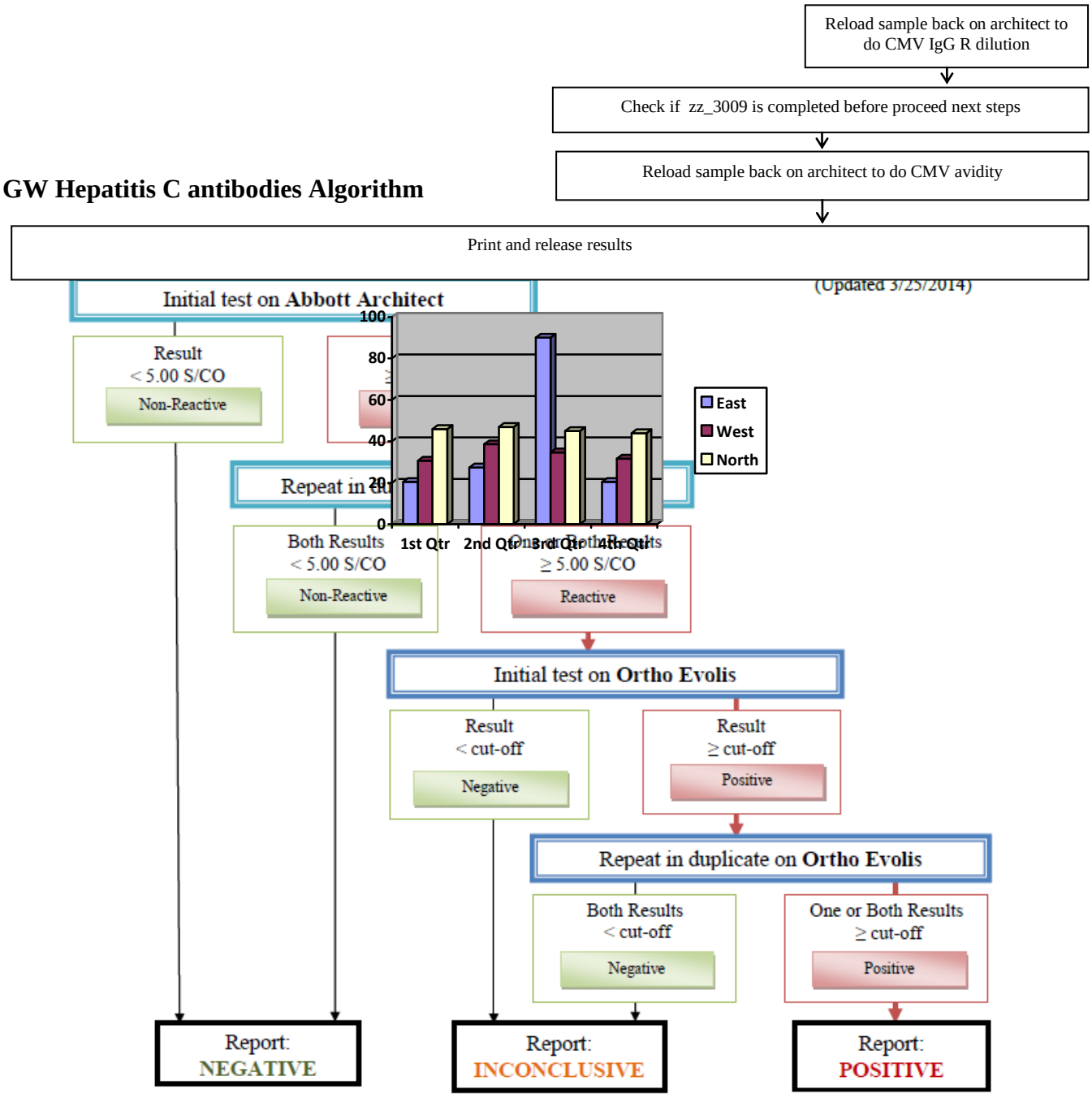
10µL specimen + 490 µL Architect Multi-Assay manual Diluent

- Repeat CMV avidity **ONLY** on architect (order **CMV G>AV** on Architect)
- Note: If CMV IgM must be repeated, use neat specimen.

GW CMV Avidity Algorithm



GW Hepatitis C antibodies Algorithm



Reporting

CMV Avidity result reporting

1) CMV Avidity≥50%, every results are autoverified except the following:

LIS order	Result	Comment
10CMR	Not Needed	Verify Result

2) CMV Avidity<50%, 2nd CMV AVIDITY <50%,
*Manually order 10CA2 in LIS

LIS order	Result	Comment
10CMA	**1st** CMV Avidity result	Verify Result
10CMR	**Repeated** CMV IgM result	Verify Result
10CA2	**2nd** CMV Avidity result	Verify Result

3) CMV Avidity<50%, 2nd CMV AVIDITY >50%, RUN ONE MORE CMV AVIDITY.
10CA2 and 10CA3 must be manually ordered in lis

- 3RD CMV Avidity <50%

LIS order	Result	Comment
10CMA	**1st** CMV Avidity result	Verify Result
10CMR	**Repeated** CMV IgM result	Verify Result
10CA2	**2nd** CMV Avidity result	Verify Result
10CA3	3RD CMV Avidity	Verify Result

- 3RD CMV Avidity≥ 50%

LIS order	Result	Comment
10CMA	**1st** High CMV Avidity result	Verify Result
10CMR	**Repeated** CMV IgM result	Verify Result
10CA2	Low CMV Avidity result	Verify Result
10CA3	Last high CMV Avidity	Verify Result

4) Leave all CMV Avidity results print-out papers on Senior bench

Anti-HCV result reporting for Architect:

Initial Anti-HCV Reactive<5.00 S/CO

LIS Code	Result	Comment
8HCA	Negative	Verify result
8HCA2	Architect value	Verify Result
8HCAE	Not processed	In the comment , type 'Test not requested' and Verify

Initial Anti-HCV≥5.00 S/CO

- Both duplicated Anti-HCV <5.00S/CO

LIS Code	Result	Comment
8HCA	Negative	Verify result
8HCA2	Negative	In the comment, type all 3HCA , Verify Result
8HCAE	Not processed	In the comment , type 'Test not requested' and Verify

- One Anti-HCV<5.00 S/CO and one Anti-HCV≥5.00 S/CO

LIS Code	Result	Comment
8HCA	Check#	Do **NOT** Verify In the comment, type all 3 HCA results separated by / (e.g.:### / ### / ###)
8HCA2	Type two repeated HCA results	Verify Result
8HCAE	Leave blank	Do **NOT** Verify

- Both duplicated Anti-HCV ≥5.00 S/CO

LIS Code	Result	Comment
8HCA	Check#	Do **NOT** Verify In the comment, type all 3 HCA results separated by / (e.g.:### / ### / ###)
8HCA2	Type two repeated HCA results	Verify Result

8HCAE	Leave blank	Do **NOT** Verify

Final reporting after 8HCAE results available from Evolis Manual

- 8COM must be ordered after initial 8HCAE result is available

Related Documents

Serology Specimen Management Manual	
Architect Cal _ Control Inventory	T:\microbiology\Virology\architect log sheet
Architect Consumable Log	
Architect Reagent Lot-Cal-QC chart	
Architect Done Storage	
GW Hemolyzed Sample Log	T:\microbiology\Virology\CMV Avidity\GW Hemolyzed Sample Log.xls

Reference

Abbott Operation Manual (201837-106).
Assay Packages inserts.

Record of Edited Revisions

Manual Section Name: Serology Manual for George Washington

Page Number / Item	Date of Revision	Signature of Approval
Transferred from Serology Manual, Policy # MI/SER/v51	2016.05.17	Dr. T. Mazzulli
Annual Review Page 9, updated reporting results for 2nd CMV Avidity result if less than 50% from release to do not release, bolded and in red font.	May 29th, 2018	Dr. T. Mazzulli
Annual Review	May 29, 2019	Dr. T. Mazzulli
Annual Review	May 29, 2020	Dr. T. Mazzulli

Full document review included in all updates. Bi-annual review conducted when no revision had been made within 2 years.

Page Number / Item	Date of Revision	Signature of Approval
Minor Formatting changes	April 12, 2021	Jessica Bourke
