TRAINING UPDATE

Lab Location:	SGMC	**Date Distributed:**	10/28/2021
Department:	Core Lab	**Due Date:**	11/28/2021

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Human Chorionic Gonadotropin, Total hCG by Atellica IM Analyzer
SGMC.C3056 v2

Description of change(s):

Section	Reason
10.4	Changed CRR upper limit to 200,000
10.6	Added DI flag for result >1,000; added extended dilution process
19	Added addenda A & B

This revised SOP is already implemented

Document your compliance with this training update by taking the quiz in the MTS system.

Technical SOP

Title	**Human Chorionic Gonadotropin, Total hCG by Atellica IM Analyzer**
Prepared by	Ashkan Chini Date: 5/3/2021
Owner	Robert SanLuis Date: 5/3/2021

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Test Code
Human Chorionic Gonadotropin, Quantitative	Atellica IM Analyzer	HCGQ

Synonyms/Abbreviations
Pregnancy test, Quant; Quant hCG; Total HCG, THCG

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The Atellica IM ThCG assay is a 2-site sandwich immunoassay using direct chemiluminescent technology, which uses constant amounts of 2 antibodies. The first antibody, in the Lite Reagent, is a goat polyclonal anti‑hCG antibody that has been affinity purified and labeled with acridinium ester. The second antibody, in the Solid Phase, is a purified mouse monoclonal anti‑hCG antibody, which is covalently coupled to paramagnetic particles. These 2 antibodies are specific for different epitopes that are present on both the free ß‑subunit and the ß‑subunit of intact hCG.
A direct relationship exists between the amount of hCG present in the patient sample and the amount of relative light units (RLUs)detected by the system

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Normal procedures for collecting and storing serum and plasma may be used for samples to be analyzed by this method.
Special Collection Procedures	N/A
Other	N/A

3.2 Specimen Type & Handling

Criteria	
Type -Preferred	Serum
-Other Acceptable	Plasma (Lithium Heparin)

Criteria	
Collection Container	Serum: Red top tube, Serum separator tube (SST) Plasma: Mint green top tube (PST)
Volume - Optimum **- Minimum**	1.0 mL 0.5 mL
Transport Container and Temperature	Collection container or plastic vial at room temperature
Stability & Storage Requirements	Room Temperature: 8 hours
	Refrigerated: 48 hours
	Frozen: Not established
Timing Considerations	N/A
Unacceptable Specimens & Actions to Take	Specimens that are unlabeled, improperly labeled, or those that do not meet the stated criteria are unacceptable. Request a recollection and credit the test with the appropriate LIS English text code for "test not performed" message. Examples: Quantity not sufficient-QNS; Wrong collection-UNAC. Document the request for recollection in the LIS.
Compromising Physical Characteristics	Gross hemolysis. Reject sample and request a recollection. Credit the test with the appropriate LIS English text code explanation of HMT (Specimen markedly hemolyzed)
Other Considerations	Allow Red Top or SST to clot completely prior to centrifugation. Before placing on system, ensure samples are free of: • Bubbles or foam • Fibrin or other particulate matter

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation. When placed onboard the analyzer, the instrument captures the date / time loaded and calculates and tracks the opened expiration.

4. REAGENTS

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
Total hCG (ThCG)	Siemens, Atellica IM, Cat. No. 10995690
ThCG DIL	Siemens, Atellica IM, Cat. No. 10995691

4.2 Reagent Preparation and Storage

Reagent	**Total hCG (ThCG)**
Storage	• Store at 2-8°C • Store in an upright position. • Protect from heat and light.
Stability	Reagents are stable onboard the system for 21 days.
Preparation	Reagent is liquid and ready to use. Before loading the reagent onto the system, mix it by hand and visually inspect the bottom of the reagent pack to ensure that all particles are re-suspended.

Reagent	**ThCG DIL**
Storage	Store at 2-8°C in an upright position.
Stability	Stable onboard the system for 28 days.
Preparation	Liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	**Supplier and Catalog Number**
Calibrator B (CAL B)	Siemens Atellica IM, Cat. No. 10995503

5.2 Calibrator Preparation and Storage

Calibrator	Calibrator B (CAL B)
Preparation	1. Add 5.0 mL of reagent grade water into Low and High Calibrator vials using a calibrated pipette. Replace cap. 2. Let the vials stand for 15–20 minutes at room temperature to allow the lyophilized material to dissolve. 3. Gently mix and invert the vials to ensure homogeneity of the material.
Storage/Stability	• Store at 2-8°C in an upright position. • **Unopened:** stable until expiration date stamped on the box. • **Reconstituted:** remains stable for 4 hours at room temperature and 28 days refrigerated.

5.3 Calibration Parameter

Criteria	**Special Notations**
Reference Material	Calibrator B (CAL B)
Assay Range	See Package Insert for specific assay ranges.

Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in mIU/mL.
Frequency	• When changing lot numbers of primary reagent packs. • At the end of the lot calibration interval (34 days), for a specified lot of calibrated reagent on the system. • At the end of pack calibration interval (28 days), for calibrated reagent packs on the system. • When indicated by quality control results. • After major maintenance or service. At the end of the onboard stability interval, replace the reagent pack on the system with a new reagent pack. Recalibration is not required, unless the lot calibration interval is exceeded.
Calibration Scheme	See Package Insert for specific calibration scheme.
Procedure	To load a new lot of reagent on IM Module: Note: Calibrate the new lot of reagent as soon as it is loaded on the instrument. If the reagent sits un-calibrated for a short period of time (24 hours) then it will not be eligible for a "Lot Calibration" and another new reagent will need to be loaded onboard. 1. From the home page of the IM Module Screen (small screen attached on the IM Module) make sure the analyzer status is in Standby. 2. On the IM Module Screen select Reagent Loader. Make sure the Reagent Drawer status is unlocked. Open the reagent drawer, load the reagent and then close it. Once the reagent is scanned, the IM Module Screen will populate message "Missing TDef for lot" next to the reagent. The Reagent Drawer status remains unlocked. 3. Both Reagent Master Curve and Calibrator Package Insert need to be scanned using the Atellica Solution's main monitor. To differentiate between the two: • Reagent Master Curve has MC TDEF printed right below the assay name. • Calibrator Package Insert has CAL printed right above the assay name. 4. To scan the Reagent Master Curve, go to Set up – Test Definition – IM Test Definition. Scan the barcode. 5. To scan the Calibrator Package Insert, go to Calibration – Calibrator Definition. Scan the barcode. 6. Re-open the Reagent Drawer and close it. This time its status should change to locked, meaning the reagent is going to be loaded onboard ready for calibration.

Procedure	Refer to the Atellica Solution Operating, QC, Calibration and Maintenance procedure for specific instructions.

5.4 Tolerance Limits

IF……	THEN……
If result fall within assay-specific specification, and QC values are within acceptable limits,	proceed with analysis
If result falls outside assay-specific specification, or QC values are out of Acceptable limits,	troubleshoot the assay and/or instrument and repeat calibration

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
InteliQ Immunoassay Plus Controls, Levels 1, 2 & 3	Bio-Rad Laboratories Cat. No. 12009948, 12009949, 12009950

6.2 Control Preparation and Storage

Control	InteliQ Immunoassay Plus Controls
Preparation	Allow to thaw at room temperature (18-25C) for approximately 60 minutes or until completely thawed. Once thawed, gently invert the tube several times to ensure homogeneity.
Storage/Stability	**Frozen**: until the expiration date if unopened at -20 to -70C **Thawed and Unopened:** 30 days at 2-8C **Thawed and Onboard:** 14 days at 2-8C Note: Stability for PSA and Folate is shorter.

6.3 Frequency

Analyze all levels of QC material after every calibration and each day of testing (notated on the QC frequency sheets posted on the instruments).

Refer to the Atellica QC Schedule and the Siemens Atellica Quick Reference Guide.

6.4 Tolerance Limits and Criteria for Acceptable QC

Step	Action
1	Acceptable ranges for QC are programmed into the instrument's Quality Control software system and Unity Real Time, and may be posted near the instrument for use during computer downtime.

Step	Action
2	**Run Rejection Criteria** • Anytime the established parameters are exceeded (if one QC result exceeds 2 SD), the run is considered out of control (failed) and patient results must not be reported. • The technologist must follow the procedure in the Laboratory QC Program to resolve the problem.
3	**Corrective Action**: • All rejected runs must be effectively addressed through corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed according to the Laboratory QC Program. Supervisors may override rejection of partial or complete runs only with detailed documentation and criteria for overrides that are approved by the Medical Director. Consult corrective action guidelines in Laboratory QC Program. Follow corrective action guidelines in the Laboratory QC Program. • Corrective action documentation must follow the Laboratory Quality Control Program.
4	**Review of QC** • QC must be reviewed weekly by the Group Lead or designee and monthly by the Supervisor/Manager or designee. • If the SD and/or CV are greater than established ranges, investigate the cause for the imprecision and document implementation of corrective actions.

6.5 Documentation

- QC tolerance limits are programmed into the instrument and Unity Real Time; it calculates cumulative mean, SD and CV and stores all information for easy retrieval.
- Quality control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.6 Quality Assurance Program

- Each new lot number of reagent or new shipment of the same lot of reagent must be tested with external control materials and previously analyzed samples. Performance of the new lot must be equivalent to the previous lot; utilize published TEA for acceptability criteria.
- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.

- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Monthly QC must be presented to the Medical Director or designee for review and signature.
- Monthly QC mean and SD are sent to Bio-Rad Laboratories for peer group comparison.
- Consult the Laboratory QC Program for complete details.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Siemens Atellica IM Analyzer

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.
- Centrifuge

7.3 Supplies

- System Fluids
- Assorted calibrated pipettes (MLA or equivalent) and disposable tips

8. PROCEDURE

Atellica IM Total hCG (ThCG) is required to perform this test.

Total hCG is performed on the Atellica IM Analyzer after the method is calibrated and Quality Controls are acceptable.

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

8.1	Instrument Set-up Protocol
1.	Perform any required instrument maintenance.
2.	Ensure that the instrument has sufficient primary and ancillary reagents.
3.	Check status of cuvettes and tips. Check waste levels. Fill or empty as appropriate.
4.	Check calibration status and re-calibrate as needed.

8.2	Specimen Testing
1.	Centrifuge the specimens.

8.2	Specimen Testing
2.	Load the sample in the Atellica rack and place the rack into the Sample Handler to initiate testing. **NOTE: If not equipped with an in-line decapper unit, samples must be de-capped prior to loading on the Atellica system
3.	Refer to the general operating procedure for detailed steps.
4.	Follow protocol in section 10.6 "Repeat Criteria and Resulting" for samples with results above the analytical measurement range (AMR). Investigate any flagged results and repeat as necessary.
5.	Append the appropriate English text code qualifier messages to any samples requiring a comment regarding sample quality and/or any other pertinent factors.

NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen.

9. CALCULATIONS

The instrument automatically calculates the concentration of Total hCG in mIU/mL.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required

10.2 Rounding

No rounding is necessary. Instrument reports results as a whole number.

10.3 Units of Measure

mIU/mL

10.4 Clinically Reportable Range (CRR)

3 – 200,000 mIU/mL

10.5 Review Patient Data

Each result is reviewed for error messages. Resolve any problems noted before issuing patient reports.

10.6 Repeat Criteria and Resulting

All repeats must replicate the original result within the total allowable error (TEa) of the assay. Refer to TEa listing for specific information.

Values that fall below or within the AMR or CRR may be reported without repeat. Values that exceed the upper ranges must be repeated.

IF the result is …	THEN…
< 3 mIU/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 3 mIU/mL
≥ 1,000 mIU/mL	**On Board Automated Dilution:** Results ≥ 1000 mIU/mL will automatically have repeat testing performed on the instrument using an onboard dilution factor of 200. No multiplication is necessary. **Note**: DI will flag "*Test Being diluted. Please wait for result*" as reminder to not release this result. See addenda A for DI screen shots.
> 200,000 mIU/mL	If the recommended dilution does not give results within the clinically reportable range, a message will display in DI stating "*Final Dilution. Release result*." Once you have the final diluted result, release the final value and reject the preliminary. Do not release both results. Report as: > 200,000 mIU/mL -REP

At the client request, values >200,000 can be further quantitated as follows:

Order and Prepare / Run dilution	
Order test: • Place a new order for **HCGQD** (HCG Diluted). HCGQD must be on its own accession number • Use the collection date/time from the original specimen • Use the requesting physician as the ordering physician **Manual Dilution**: Make a one thousand-fold (1:1000) dilution (refer to addenda B for detailed instructions). **Diluent**: Atellica IM ThCG DIL To program the dilution: • Go to Patient Order tab • Click Create Patient Orders tab • Enter accession number and press Enter • Select the assay (test) from the list displayed • The test will default to x1 (undiluted) – uncheck this • Enter the manual dilution factor 1,000 in the appropriate field • Press Enter and print barcode The barcode has the dilution factor embedded in it and the instrument will do the calculation automatically. No multiplication is required on the user end. Label tube with barcode and load.	
IF the result is …	**THEN…**
<1,000,000 mIU/mL	Report the value obtained.
>1,000,000 mIU/mL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 1,000,000 mIU/mL - REP" Bring to the attention of Tech in Charge (TIC) or Group Lead to check for integrity issues prior to release of results.

Message	Code
Verified by repeat analysis	Append –REP to the result.

11. EXPECTED VALUES

11.1 Reference Ranges

Age	Non- Pregnant Female	Male
All	1 - 3 mIU/mL	0 - 2 mIU/mL

11.2 Critical Values

None established

11.3 Standard Required Messages

Each result has the following interpretation automatically added to the report by the LIS:

HCG levels with Gestational Age

Gestational Age	hCG mIU/mL (IU/L)
0.2-1 week	5-50
1-2 weeks	50-500
2-3 weeks	100-5,000
3-4 weeks	500-10,000
4-5 weeks	1,000-50,000
5-6 weeks	10,000-100,000
6-8 weeks	15,000-200,000
2-3 months	10,000-100,000

12. CLINICAL SIGNIFICANCE

Human chorionic gonadotropin (hCG) is a heterodimeric (α plus s) sialoglycoprotein hormone produced by the placenta soon after a fertilized ovum implants into the uterine wall. Presence of hCG in serum shortly after conception, followed by a rapid rise in concentration, makes it an excellent marker to confirm and monitor a pregnancy. Physiologically, hCG appears to maintain the corpus luteum and support the endometrium. Serum and plasma hCG concentrations peak during the first trimester, then decrease and plateau during the remainder of pregnancy, circulating as the intact heterodimer in the blood of healthy women who have an uncomplicated pregnancy.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/modified
- **Validated Test Modifications:** Plasma sample types validated

The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report slip containing such error messages should be held for follow-up.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

3 – 1,000 mIU/mL

14.2 Precision

Material	Mean mIU/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Serum 1	2.4	0.2	8.8
Serum 2	12.6	0.4	2.8
Serum 3	787	14	1.8
Control 1	6.8	0.3	4.5
Control 2	23.4	0.6	2.6
Control 3	202.1	3.6	1.8

14.3 Interfering Substances

HIL Interference:
Interfering substances at the levels indicated in the table below were tested in accordance with CLSI Document EP07-A2.

Substance tested	Substance Concentration	mIU/mL	Bias %
Hemoglobin	1000 mg/dL	5.6	8.9
Bilirubin (conjugated)	40 mg/dL	6.1	-0.3
Bilirubin (unconjugated)	40 mg/dL	6.0	0.3
Lipemia Intralipid®	3000 mg/dL	6.1	2.0

14.4 Clinical Sensitivity/Specificity/Predictive Values

Detection Capability
The assay is designed to have a limit of blank (LoB) ≤ 2.0 mIU/mL, a limit of detection (LoD) ≤ 4.0 mIU/mL, and a limit of quantitation (LoQ) ≤ 6.0 mIU/mL.
The LoB of the Atellica IM ThCG assay is 1.5 mIU/mL. The LoD for the Atellica IM ThCG assay is 1.7 mIU/mL. The LoQ of the Atellica IM ThCG assay is 2.6 mIU/mL.

Patient samples with high hCG levels can cause a paradoxical decrease in RLUs (high-dose hook effect). In this assay, hCG levels as high as 1,000,000 mIU/mL will assay greater than 1,000 mIU/mL.

15. SAFETY

Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards.

16. RELATED DOCUMENTS

1. Atellica Solution Operating, QC, Calibration and Maintenance procedure
2. Laboratory Quality Control Program
3. Atellica Solution QC Schedule
4. Laboratory Safety Manual
5. Safety Data Sheets (SDS)
6. Atellica Solution Limits Chart
7. Retention of Record and Materials (Lab Policy)
8. Atellica Solution System Error Messages Chart
9. Centrifuge Use, Maintenance and Function Checks (Lab policy)
10. Specimen Acceptability Requirements (Lab policy)
11. Repeat Testing Requirement (Lab policy)
12. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
13. Current package insert of Total hCG Reagent

17. REFERENCES

1. Package Insert, Total hCG Reagent, Siemens Healthcare Diagnostics Inc., 02/2020
2. Package Insert, Calibrator B (CAL B), Siemens Healthcare Diagnostics Inc., 08/2019
3. Package Insert, InteliQ Immunoassay Plus Controls, Bio-Rad Laboratories, 12/2020

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
1	10/27/21	10.4	Changed CRR upper limit to 200,000	H Genser	R SanLuis
1	10/27/21	10.6	Added DI flag for result >1,000; added extended dilution process	H Genser	R SanLuis
1	10/27/21	19	Added addenda A & B	L Barrett	R SanLuis

19. ADDENDA

A. Preliminary vs Final Diluted Value in DI (Atellica Only)
B. Simple Steps for Manual Dilution

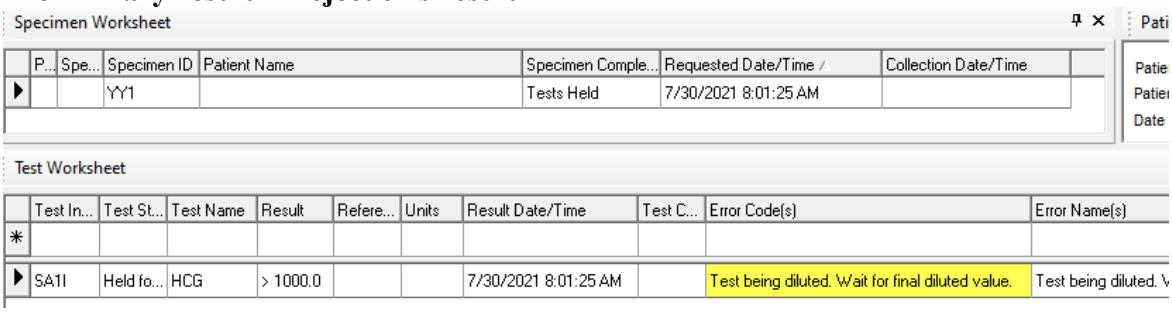
Addenda A

Preliminary vs Final Diluted Value (Atellica Only)

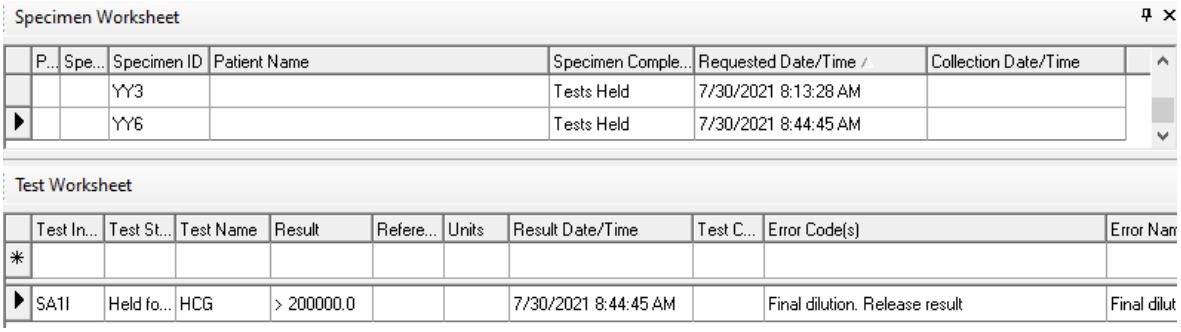
If the Atellica has resulted an HCG test that is not yet final (ie. result that needs to be diluted), the result will transmit to DI with an error code of "Test being diluted. Wait for final diluted value."
The instrument will perform an auto-dilution on that test.

If after doing the recommended dilution and the result is still not within the clinically reportable range, a message will display that says "Final Dilution. Release result." Once you have the final diluted result, release the final value and reject the preliminary. Do not release both results.

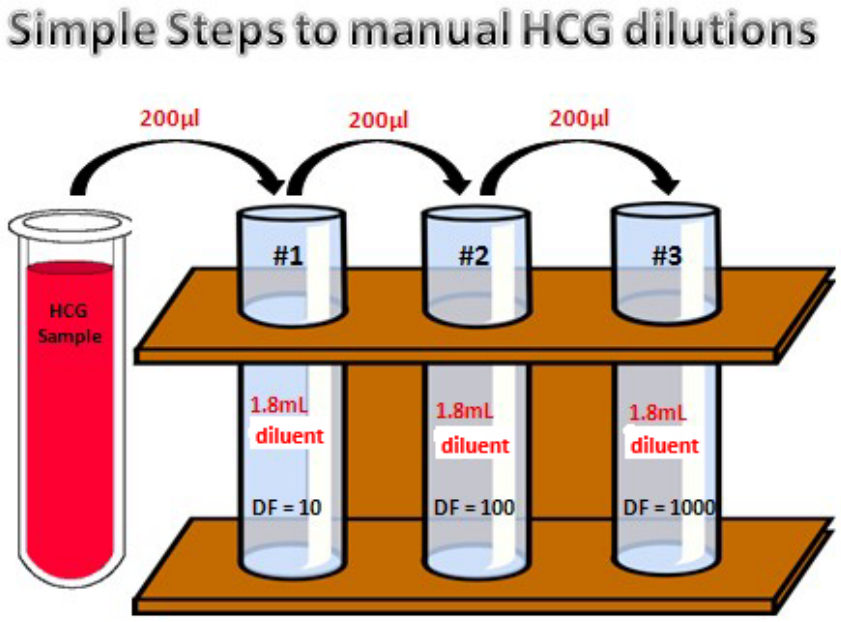
Preliminary result – Reject this result



Final diluted result – Release this result



Addenda B



Materials required for HCG serial dilution

- Transfer plastic pipettes
- Three 10mL glass test tubes
- Three automatic pipettes (200µL micropipette, 1mL pipette and 2mL pipette) with their appropriate pipette tips.
- 10mL diluent

Procedure

1. Set up three glass test tubes each labeled 1, 2 and 3 and transfer 2mL of diluent in each glass tube.
2. Remove 200µL of diluent from each test tube and discard.
3. Transfer 200µL of patient plasma/serum to tube #1 (Creating a 1:10 sample dilution)
4. Use a transfer plastic pipette and mix thoroughly (at least five to six times) to create a homogenous mixture.
5. Pipette 200µL of the mixture from tube #1 and transfer to Tube #2 and repeat step 4.
6. Pipette 200µL of the mixture from tube #2 and transfer to Tube #3 and repeat step 4.
7. Transfer the mixture from test tube #3 to a limited sample cup labeled with patient name and accession number. Proceed with the manual dilution testing procedures using 1000 dilution factor.

NOTE

- You can perform the same serial dilutions with the same dilution factor by decreasing the volume size in each step when there is a limited patient sample.
- DO NOT forget to discard the pipette tips after each transfer to avoid a sample carryover from the previous dilution.