

FMH QuikQuant™

Rapid Assay for Fetomaternal Hemorrhage Quantification



QQF-100



100 tests



package insert



CE

In Vitro Diagnostic medical device

Intended Use

FMH QuikQuant™ is intended for use in hospital clinical and reference laboratories by trained medical technologists or similar individuals having experience in test methods for fetomaternal hemorrhage.

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Summary and Principle

The most important use of fetal RBC detection is the evaluation of fetomaternal hemorrhage (FMH) [1-4]. FMH occurs normally throughout pregnancy in minute amounts with increasing volumes during the later stages of gestation [5]. If there is a significant difference in the RBC antigenicity between the fetus and mother, this can result in allosensitization of the maternal immune system either before or after parturition. The maternal antibodies to the fetal RBC antigens may be clinically silent or cause life-threatening autoimmune sequelae for the current or subsequent pregnancies (e.g.: erythroblastosis fetalis or early abortion). Such sensitization can occur with any RBC antigen mismatch, but the highest frequency and profound clinical consequences occur with Rh or D-antigen mismatches. Detection and enumeration of Fetal RBCs is an essential part of the management of those patients with FMH treated with Rh immune globulin (RhIG) preparations [6]. The use of Rh immune globulin prophylaxis is a universal practice, but dosing amounts and schedules have regional variations [7,8]. Hence, the sensitivity and specificity of detection assays for FMH is a critical factor in therapeutic efficacy and subsequent clinical outcome.

The most widely used assay for FMH detection has been the visual microscopic counting Kleihauer-Betke (KB) method, which is based upon differences in solubility properties in acid conditions of fetal hemoglobin (HbF) from adult hemoglobin [9]. While the KB method is easily performed by most clinical laboratories, it lacks sensitivity and exhibits poor reproducibility or precision (CVs of 50-100%) [10,11]. Flow cytometric methods have been developed using the antigenic differences or quantitative assessment of fetal hemoglobin (HbF) to distinguish fetal RBCs from adult RBCs. These methods are more precise and less subjective [12-22]. Nonetheless, many laboratories have continued to use the KB method due to the limited availability of flow cytometry.

FMH QuikQuant™ is a flow cytometric method for FMH detection and quantitation. The assay uses an anti-Hemoglobin F monoclonal antibody and propidium iodide reagent in a no-wash technique requiring about 45 minutes to complete. With less than 15 minutes of technologist time needed, it is both, more efficient to use than the KB assay, as well as more sensitive and precise.

Application

The laboratory determination of the level of fetal cells in maternal circulation remains an important tool in the obstetrical management of women with suspected uterine trauma and in the proper dose administration of Rh immune globulin.

Product Components

- 1.0 mL FMH QuikQuant™ antibody reagent for flow cytometry (100 tests)
- 40 mL *Intra-Cell*™ permeabilization solution – provided as 10X concentrate
- 40 mL FMH QuikQuant™ buffer solution – provided as 10X concentrate

Reagents and Materials Required, but not Included

- 12 x 75 mm disposable polystyrene tubes with rack
- Pipette tips, (1-200 µL) and (200-1000 µL)
- Adjustable volume pipettes (0-200, and 200-1000 µL)
- Phosphate buffered saline (PBS) or Dulbecco's PBS Sigma #P3813 or CellGro #21-031-CM
- Glutaraldehyde (0.03% ± 0.015%)
 - 8% glutaraldehyde for dilution Sigma #G7526 or Polysciences #00216A
- **OR**
- 25% glutaraldehyde for dilution Sigma #G5882 or Polysciences #01909
- Reagent grade water, filtered is preferable
- 0.2 µm vacuum filter set up (optional, but recommended)
- FETALtrol™ (Diagnostics) or home brew cord blood for 3 levels of controls for fetomaternal hemorrhage assays
- Vortex mixer
- Blood bank cell washer or centrifuge capable of achieving 600 x g
- Multiparametric flow cytometer, capable of at least 3 fluorescence parameters
- Software for analysis of FCS format listmode files

Reagent Preparation

1 PBS

- Dulbecco's PBS 1000 mL CellGro #21-031-CM or equivalent
- OR**
- Phosphate buffered saline powder 1 packet Sigma #P3813 or equivalent
- Reagent grade H₂O QS to 1000 mL
- Mix well any solution prior to pH or filtration
- Filter using 0.2 µm-pore vacuum filter, if available, to remove particulate debris
- Adjust pH to 7.4 when solution reaches room temperature (20-25°C)
- Store in refrigerator at 2-8°C
- Expiration to be determined by laboratory standard procedure. Using sterile / filtered water as diluent will extend shelf life to 30 days at 2-8°C.

2 FMH QuikQuant™ buffer **working solution – equivalent to PBS with BSA (0.5%)**

- Reagent Grade H₂O 9 parts
- FMH QuikQuant™ buffer concentrate 1 part
- For example: 9 mL H₂O and 1 mL BSA concentrate combined***
- Mix well
- Filter using 0.2 µm-pore vacuum filter, if available to remove particulate debris
- Adjust pH to 7.4 when solution reaches room temperature (20-25°C)
- Store in refrigerator at 2-8°C
- Expiration: to be determined by laboratory standard procedure. Using sterile / filtered water as diluent will extend shelf life to 30 days at 2-8°C.

3 Working dilution of glutaraldehyde: 0.03%

Note: As purity of glutaraldehyde may vary with manufacturer or lots, concentrations between 0.015 – 0.045% glutaraldehyde should be evaluated for optimization of separation of adult and fetal RBC staining with assay (2 logs or more separation desirable).

Store **stock** glutaraldehyde in freezer (ampules may be aliquoted into polypropylene microtubes and refrozen) or in refrigerator following manufacturer's recommendation. Make fresh working solution each day of use

- **8%** EM grade or grade I glutaraldehyde stock solution 94 µL Sigma #G7526 or Polysciences #00216A
- PBS (pH 7.4) 25 mL (see PBS above)
- OR**
- **25%** EM grade or grade I glutaraldehyde stock solution 30 µL Sigma #G5882 or Polysciences #01909
- PBS (pH 7.4) 25 mL (see PBS above)
- Mix well and hold in refrigerator (2-8°C) up to 2 hours prior to use
- Expiration: discard unused diluted glutaraldehyde / See manufacturer's container for expiration of stock glutaraldehyde.

4 *Intra-Cell*™ for cell permeabilization

- *Intra-Cell*™ reagent concentrate must be held at room temperature (20-25°C) and mixed well prior to dilution to avoid precipitated solids
- Prepare 1:10 dilution working solution from *Intra-Cell*™ reagent concentrate, for example
 - *Intra-Cell*™ concentrate 5 mL
 - Reagent grade water (H₂O) 45 mL
- Mix well and store **working** solution (*Intra-Cell*™ diluted 1:10) in refrigerator (2-8°C).
- Expiration: working solution expires at 30 days or at detection of any cloudiness in the solution. Stock solution may be used until labeled outdate.

Specimen

EDTA whole blood (or other anti-coagulated samples)

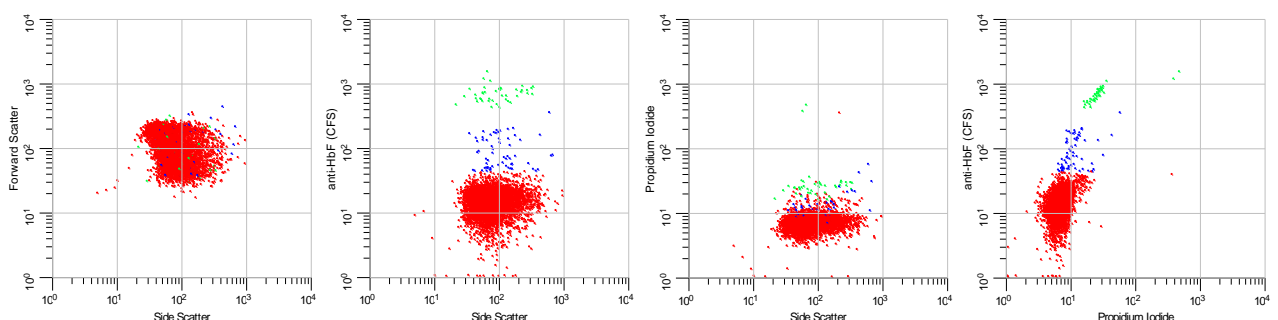
- Refrigerate specimen if testing is not performed within 4 hours post collection
- Specimens may be held refrigerated for 72 hours prior to testing [1].

Specimen Preparation

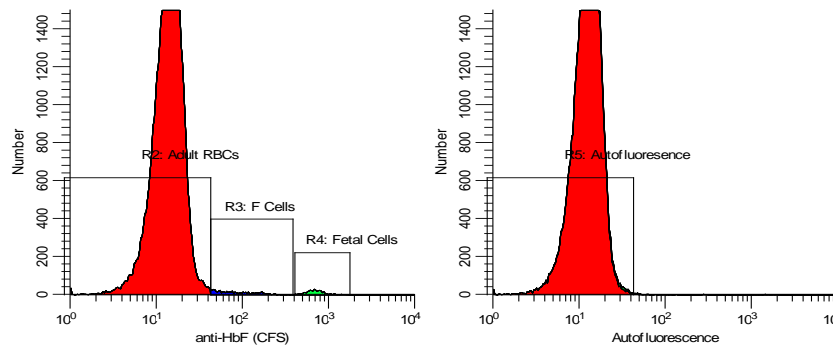
- 1 Make a 1:20 dilution of FETALtrol™ or EDTA anti-coagulated blood using PBS with 0.5% BSA or FMH QuikQuant™ buffer working solution.
- 2 Place 10 µL of the diluted cells into a 12 X 75 mm polystyrene plastic tube.
- 3 Add 0.75 mL glutaraldehyde (0.03% glutaraldehyde in PBS **without** BSA) to tube.
- 4 Vortex upon addition of glutaraldehyde to cells and intermittently during incubation at room temperature for 10 minutes. Avoid RBC clump formation by ensuring cells remain in suspension during this fixation step.
- 5 Add 1.5 mL of *Intra-Cell*™ working solution to each tube and incubate at room temperature for 10 minutes.
- 6 Vortex upon addition of the *Intra-Cell*™ solution and at least twice during incubation, again minimizing cell aggregates.
- 7 Spin for 60 seconds with cell washer and decant for 5 seconds. Alternatively centrifuge at least 5 minutes in a centrifuge at 600 x g, and then decant supernatant.
- 8 Vortex tube for at least 15 seconds to completely disperse pellet.
- 9 Add 10 µL of FMH QuikQuant™ antibody reagent, followed by 40 µL FMH QuikQuant™ buffer working solution.
- 10 Incubate for 10 minutes at room temperature in the dark.
- 11 Add 2.0 mL of FMH QuikQuant™ buffer working solution, vortex and incubate at room temperature for 30 seconds. **Note: To facilitate mixing without spilling, we suggest adding 1 mL, vortexing gently, then adding the other 1 mL and mixing again.**
- 12 Spin for 60 seconds with cell washer and decant for 5 seconds. Alternatively centrifuge at least 5 minutes in a centrifuge at 600 x g, and then decant supernatant.
- 13 Add 1.0 mL FMH QuikQuant™ buffer working solution, mix tube thoroughly, and avoid exposure to light. Run on flow cytometer collecting a minimum of 100,000 RBCs for analysis.

Flow Cytometer Set-Up

- 1 Select 2 blood samples, one adult blood with a high WBC and either one adult blood spiked with washed, ABO compatible cord cells or FETALtrol™ Level 3. (All red blood cells must be washed twice and resuspended in PBS/BSA prior to mixing or spiking to remove all hemagglutinins that may cause clumping).
- 2 Stain according to FMH QuikQuant™ assay staining procedure.
- 3 Use the following steps to set up the cytometer protocol:
 - a) Draw the following:
 - **Two-parameter histograms:**
 - FSC vs SSC (log-log)
 - Anti-HbF (FL1) vs SSC-log
 - Propidium iodide (FL3) vs SSC-log
 - Propidium iodide (FL3) vs anti-HbF (FL1)



- **Single parameter histogram(s):**
 - Number vs anti-HbF (FL1)
 - Number vs autofluorescence (FL2) (optional)



And a **Results box** showing gated RBC events, adult RBCs, adult F-cells, and Fetal RBCs (autofluorescence and nucleated cells are optional)

Id1: QQ_FMH-034_B.14.001				
Id2: 19Jan2009				
	Alias	Region	%Gate	MedianX
R1	RBC Gate	49905	99.81	
R2	Adult RBCs	48987	97.97	13.82
R3	F-Cells	616	1.23	74.59
R4	Fetal Cells	391	0.78	684.98
R5	Autofluorescence	49897	99.79	12.51
R6	Nucleated cells	35	0.07	64.47

- b) While running the tube containing the **stained adult / cord blood mixture** (or **FETALtrol™ Level 3**), adjust FS-log and SS-log so that the RBC population lies midscale on both axes.
- c) Adjust threshold on FS to eliminate unwanted events (platelets, cell debris) having a lower signal than the blood red cell population.
- d) Adjust FL1 (anti-HbF) and FL3 (propidium iodide) so that the red blood cell population is in the first decade of both parameters and the entire peak can be visualized on the histograms. While propidium iodide is a specific marker for nucleated cells, autofluorescence can be used in setting regions when enumerating F-cells [22].
- e) While running the tube containing the **stained sample with the high WBC**, adjust FL3 – FL1 Compensation so that the (stained) RBCs along the FL1 axis will fall mostly within the first decade on the FL3 axis, but not having >1% of RBCs at the baseline of the FL1 signal.
- f) Place a gating region around the red blood cells, **excluding RBC aggregates**, cellular debris and nucleated cells (propidium iodide positive events). Be sure the single parameter histogram is based on this gate (G1 = R1). Set analysis regions for adult RBCs, adult F-cells, and Fetal RBCs (see below for adjustment of regions) on the single parameter histogram of the anti-HbF parameter.
- g) Name and save the instrument settings and the protocol template with the acquisition protocol also set to collect at least 50,000 events in a list mode file with all parameters (FS, SS, FL1, FL2 and FL3).

Listmode File Analysis

Analysis of list mode files should be performed as shown in the histograms above. A gating region should be used to exclude nucleated cells from analysis, which is recommended to be a side scatter vs. propidium iodide plot. Additionally gating strategies should include a means of exclusion of RBC aggregates from analysis using a FALS vs Side Scatter gate. Excessive RBC aggregates (>1%) may cause a significant over reporting of the Fetal RBC percentage since aggregates cause the true denominator to be under counted. As shown above it is also recommended that regions of analysis be created on a single parameter plot of anti-HbF using three regions corresponding to adult RBCs, adult F-cells, and fetal RBCs. Turning off of any autoscaling function of the analysis software will allow for better visualization of the Fetal RBC population. Alternatively two parameter displays of anti-HbF expression vs. autofluorescence may also be an effective means of defining the region of Fetal RBC identification. A consensus method for setting the regions for adult RBCs and adult F-cells has not been established, although we have proposed one approach using the autofluorescence signal [23]. The fetal RBC analysis region should be set first using the control samples in order to set the region of analysis either side of the fetal RBC peak on the high control specimen (typically 1-2% Fetal RBCs).

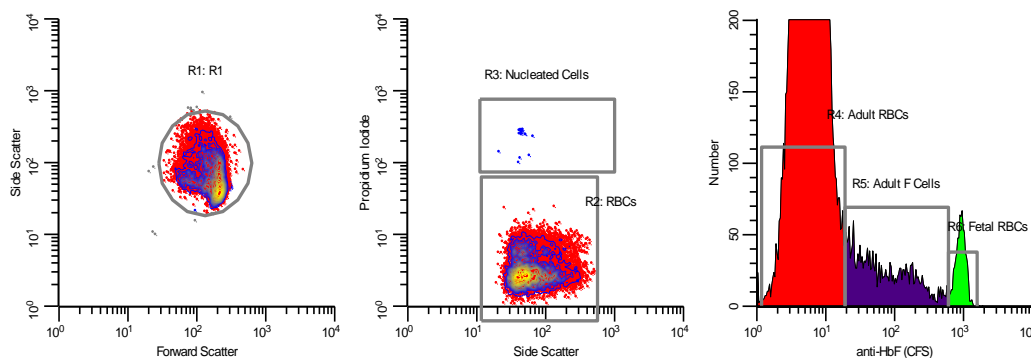


Figure 1. Data analysis should include gating to exclude RBC aggregates (R1 gate) and nucleated cells (R3 gate), then enumeration of adult RBCs (R4 region), adult F-cells if desired (R5 region), and fetal RBCs (R6 region).

Assay Quality Control

It is imperative that multi-level assayed control samples be used as a means of assessing both the procedure and the analysis. Low staining intensity, cell damage due to improper concentration of the fix and/or perm solutions, increased F cell levels, and improper compensation and suboptimal gating can seriously impact the validity of the generated results. There are two major sources of quality control samples:

1. **FETALtrol™** - Stabilized blood control product is a convenient alternative to "home brew" controls. It contains vials of negative, low level (~0.15% fetal RBCs) and high level (~1.5% fetal RBCs) positive controls and has a 3-month shelf life. This product is used exactly as whole blood in this procedure and is cleared by the U.S. FDA as an *in vitro* diagnostic control.
2. **Home brew or in-house prepared spiked blood samples** – mixtures of ABO matched fetal or umbilical cord blood with adult blood. Ideally there should be high, low, and negative controls with validated values for Fetal RBCs.

Handling and Storage

Store FMH QuikQuant™ antibody reagent vials upright, tightly capped, at 2-8°C when not in use. Store *Intra-Cell*™ Concentrate upright, tightly capped, **at room temperature**. Avoid unnecessary cycles of warming and cooling of all reagents. Protect product from freezing, from temperatures above 30°C and from prolonged time at room temperature (18-25°C), excluding the *Intra-Cell*™ Concentrate, or exposure to light. Store FMH QuikQuant™ buffer concentrate upright and at room temperature (18-25°C).

Warning

The FMH QuikQuant™ antibody reagent and FMH QuikQuant™ buffer concentrate contains sodium azide (<0.1% w/v). This chemical is a toxic and dangerous compound when combined with acids or metals. Handle with appropriate care. Solutions containing sodium azide should be disposed of properly.

Manufacturing Quality Control

The performance and specificity of reagents contained in this kit are tested using IQ Product's in-house quality control methods. Manufacturing of this product is done using quality system and manufacturing production guidelines in compliance with FDA QSR and EN ISO 13485.

Product Limitations

The following clinical conditions may result in an increased level of HbF due to elevated levels of adult F-cells and should not be confused with a fetomaternal hemorrhage [22-24]:

- Severe anemia
- Hereditary persistence of HbF
- Thalassemia
- Sickle cell disease, especially when under therapy with hydroxyurea, butyrate or other drugs that elevate HbF

Potential Pitfalls

- Inadequate staining and poor separation are usually a result of improper fixation and/or permeabilization. The glutaraldehyde must be properly stored and diluted just prior to use in the fixation step. If the cells are not adequately fixed by the glutaraldehyde, they will lyse when the *Intra-Cell*™ is added. If the *Intra-Cell*™ step is not performed properly, the conjugated HbF antibody will not be able to reach its target within the cell [24].
- Instrument setup or compensation settings are not optimized.
- Failure to use a region for defining fetal cells based on positive control, such as FETALtrol™.
- Poor mixing and/or the use of glutaraldehyde of a concentration stronger than optimal may cause aggregates, which often is detected by the presence of cell clumping and higher than expected results for the FETALtrol™ specimens.
- Failure to dilute the 10X *Intra-Cell*™ permeabilization solution concentrate will cause clumping or agglutination (similar in gross appearance to cold agglutinins or rouleaux) that will adversely affect the accuracy of the values obtained.
- Unfiltered solutions may contain microparticles that would be 'seen' and counted by the instrument. This may reduce the number of usable events (RBCs) in the total count and make analysis difficult or adversely affect the accuracy of the values obtained.
- Post-transfusion samples with immunologically mediated cell aggregates may yield falsely elevated Fetal cell values. This problem can be avoided by excluding aggregates.
- Failure to exclude autofluorescent nucleated cells during analysis of data may give a false elevation in fetal red blood cell level.

Expected Values and Their Derivation

Each laboratory should establish an acceptable reference range(s) for fetomaternal hemorrhage assays. The laboratory mean of the fetal RBC results for healthy non-pregnant blood samples is anticipated to be $\leq 0.06\%$. Adult F cell levels are generally not reported values, but the literature suggests most samples will be $< 5\%$ F-cells [25]. Normal apparently healthy laboratory donors were studied at three sites. These results should serve as guidelines. Every laboratory should establish its own reference ranges.

Performance Characteristics

FMH QuikQuant™ - Flow Cytometry Version - Sensitivity Study						
Expected Fetal RBCs%	Replicate 1	Replicate 2	Mean	Std Dev	CV	P Value
0.00	0.01	0.02	0.02	0.01	33.3%	1.00000
0.02	0.02	0.02	0.02	0.00	0.0%	0.42265
0.04	0.04	0.04	0.04	0.00	0.0%	0.03775
0.07	0.06	0.07	0.07	0.01	7.7%	0.01942
0.10	0.12	0.09	0.11	0.02	14.3%	0.02951
0.15	0.14	0.15	0.15	0.00	3.5%	0.00295
0.17	0.19	0.18	0.19	0.01	2.7%	0.00173
0.18	0.13	0.19	0.16	0.03	18.8%	0.04129
0.37	0.34	0.39	0.37	0.03	6.9%	0.00526
0.64	0.58	0.57	0.58	0.01	0.9%	0.00016
0.92	0.83	0.81	0.82	0.01	1.2%	0.00019
1.19	1.19	1.08	1.14	0.05	4.9%	0.00242
1.46	1.33	1.50	1.42	0.09	6.0%	0.00368
1.65	1.51	1.74	1.63	0.12	7.1%	0.00507
1.83	1.90	1.80	1.85	0.05	2.7%	0.00075

Table 1. Determination of assay sensitivity was performed by dilution studies of FETALtrol™ mixtures (high, low, and negative levels) followed by replicate measurements on a Becton Dickinson FACScan instrument. As shown above, samples with as low as 0.04% fetal red blood cells could be significantly ($P < 0.05$) distinguished from samples lacking fetal cells.

FMH QuikQuant™ - on FACScan - Precision Study					
Replicate	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
1	0.02	0.17	0.68	0.76	1.72
2	0.02	0.16	0.75	0.79	1.70
3	0.01	0.17	0.70	0.78	1.71
4	0.02	0.16	0.68	0.82	1.69
5	0.01	0.17	0.70	0.94	1.69
6	0.01	0.18	0.71	0.80	1.75
Mean	0.02	0.17	0.70	0.80	1.71
Std Dev	0.01	0.01	0.02	0.03	0.02
CV	33.3%	4.1%	3.4%	3.3%	1.2%

Table 2. Determination of assay imprecision was performed by studies of FETALtrol™ mixtures followed by replicate measurements on a Becton Dickinson FACScan instrument. As shown above, samples with as low as 0.17% fetal red blood cells have a coefficient of variation (CV) of $< 5\%$.

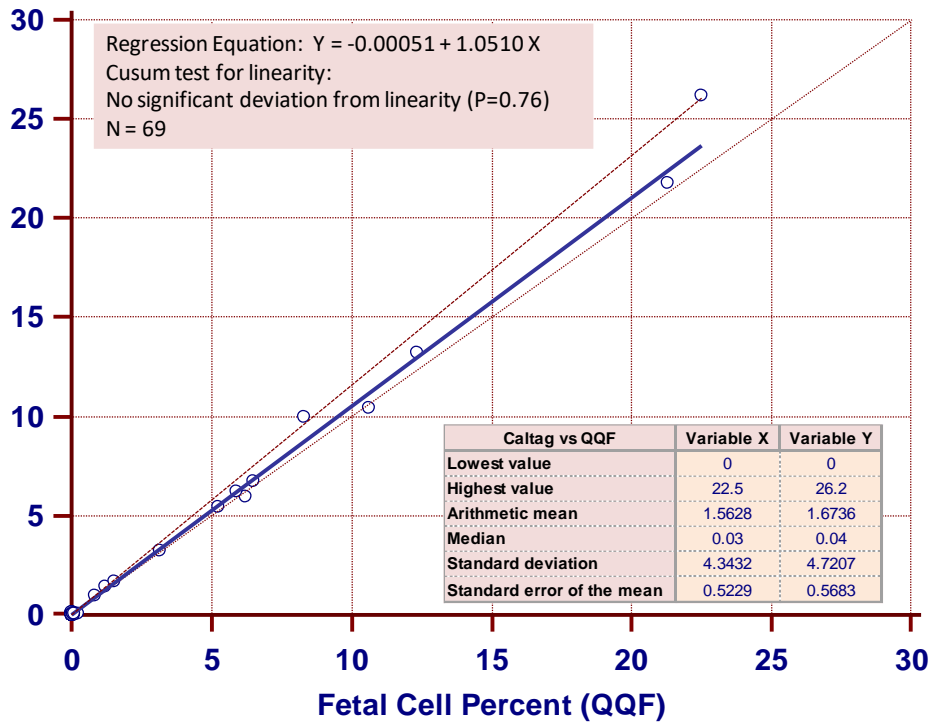


Figure 2. Determination of assay linearity was performed by Passing & Bablok regression between fetal RBC percentage determined by the flow cytometric Caltag Fetal Hgb and FMH QuikQuant™ (QQF) assays.

Regulatory Status

At this time, the FMH QuikQuant™ is registered as “in vitro diagnostic medical device” in the countries that belong to the European Community. In all other countries it should be labeled “for research use only”. Regulatory status by U.S. Food and Drug Administration has not yet been determined.


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Warranty

Products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, which extend beyond the description on the label of the product. IQ Products BV is not liable for property damage, personal injury, or economic loss caused by the product.

Explanation of used symbols [26]

	Consult instructions for use
	Catalogue number
	Sufficient for
	In Vitro Diagnostic medical device
	Caution, consult accompanying document
	Keep away from (sun)light
	Biological risks
	Temperature limitation (°C)
	For Research Use Only
	Batch code
	Use by yyyy-mm-dd
	Manufacturer
	Authorized Representative in the European Community

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