

# Quant-Rho<sup>®</sup> FITC Anti-D (Monoclonal) For *in vitro* labelling of RhD positive cells for analysis by flow cytometry

This insert refers to product Z499U

## INTRODUCTION

The development of anti-D antibodies generally results from fetomaternal haemorrhages (FMH) occurring in RhD negative women who carry a RhD positive fetus. Post-delivery immunoprophylaxis using anti-D Immunoglobulin (anti-D Ig) was approved in the US in 1968 and the UK in 1969<sup>(2)</sup>. To ensure appropriate anti-D prophylaxis, BC6H (British Committee for Standards in Haematology) Guidelines for the estimation of fetomaternal haemorrhage<sup>(1)</sup> and AABB standards<sup>(5)</sup> recommend confirmation of the volume of the fetal bleed, which may be attributed to a variety of causes including fetal trauma, various obstetrical emergencies and placental trauma. Quant-Rho<sup>®</sup> FITC Anti-D provides a rapid method for the determination of the total number of fetal RhD positive cells in a maternal RhD negative sample.

A fluorescein conjugated anti-D antibody is applied to a sample of maternal blood taken within two hours of delivery<sup>(2)</sup> and the number of cells labelled determined by flow cytometry. By calculation as described by Mollison (1972)<sup>(4)</sup>, see section Calculation of FMH describing formula, the extent of the individual event is confirmed.

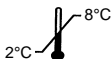
## INTERPRETATION OF LABEL SYMBOLS



Batch code



Use by (YYYY-MM-DD)



Storage temperature limitation (2°C–8°C)



*In vitro* diagnostic medical device



Consult instructions for use



Harmful



Manufacturer

## INTENDED PURPOSE

This FITC anti-D is for the estimation of FMH by *in vitro* labelling of RhD positive cells for analysis by flow cytometry.

## INDICATIONS FOR USE

Quant-Rho<sup>®</sup> FITC Anti-D should be used when the presence of fetal cells in the maternal circulation is suspected, resulting from fetomaternal haemorrhage (FMH), which may be attributed to a variety of causes including fetal trauma, various obstetrical emergencies and placental trauma.

## REAGENT DESCRIPTION

The main component of the *in vitro* diagnostic reagent Quant-Rho<sup>®</sup> FITC Anti-D is derived from the *in vitro* culture of the immunoglobulin secreting heterohybridoma cell line LDG 76. The purified monoclonal antibody is conjugated to fluorescein isothiocyanate and further purified to remove unreacted dye.

This reagent is presented as a 5ml volume. The formulation also contains 1g/l sodium azide as a preservative, buffer salts, bovine serum albumin (BSA) and sodium chloride.

## STORAGE CONDITIONS

This reagent should be stored at 2°C – 8°C. May be at room temperature (15°– 30°C) while in use. Do not use if turbid. Do not dilute. This reagent is stable until the expiry date stated on the product label.

## PRECAUTIONS FOR USE AND DISPOSAL

This reagent contains 0.1% sodium azide (EC No.247-852-1) and is classified as harmful (Xn). R22 Harmful if swallowed. Sodium azide may react with lead and copper plumbing to form explosive compounds. If discarded into sink, flush with a large volume of water to prevent azide build-up.

**Caution: Source material from which this product is derived was found non-reactive for HBsAg, Anti-HIV 1/2 and Anti-HCV. No known test methods can offer assurance that products derived from human blood will not transmit infectious disease. Appropriate care should be taken in the use and disposal of this product.**

This reagent is for *in vitro* professional use only.

## SPECIMEN COLLECTION AND PREPARATION

Specimens should be collected by aseptic technique with EDTA within two hours of delivery<sup>(2)</sup> prior to administration of therapeutic anti-D. The specimen should be tested as soon as possible after collection. If testing is delayed, the specimen should be stored at 2°C - 8°C. The sample should be processed and results reported in sufficient time to ensure that if necessary a supplementary dose of anti-D can be given within 72 hours of the delivery or sensitizing event<sup>(1)</sup>. Blood specimens exhibiting gross haemolysis, contamination or containing clots should not be used.

## LIMITATIONS OF USE

This test has the capacity to quantify FMH only in cases of RhD incompatibility.

Weak D expressions may not be detected.

In cases of ABO incompatibility between mother and child, the natural ABO antibodies of the mother may destroy fetal cells in the maternal circulation before testing is performed.

## TEST PROCEDURES

### General Information

The device is to be used by the methods as described below by trained laboratory professionals skilled in flow cytometric analysis.

This reagent has been standardised for use by the techniques described below and therefore its suitability for use in other techniques cannot be guaranteed.

## MATERIAL PROVIDED

*In vitro* diagnostic reagent Quant-Rho<sup>®</sup> FITC Anti-D (Monoclonal) for *in vitro* labelling of RhD positive cells for analysis by flow cytometry.

## ADDITIONAL MATERIALS REQUIRED

- Isotonic saline, 0.85-0.9% sodium chloride.
- RhD negative reagent red cells.
- RhD positive reagent red cells.
- Suspension (sheath) fluid as recommended by the manufacturer of the flow cytometer in use or filtered saline.
- Reaction tubes appropriate to the centrifuge and flow cytometer in use.
- Flow cytometer.
- Centrifuge set at a time and speed appropriate for the centrifuge used that allows separation of cells.
- Pipette capable of delivering 50-200µl.
- Pipette capable of delivering 1.5ml.
- Pipette tips.
- 37°C Incubator.

## RECOMMENDED TECHNIQUE

### Sample Preparation

1. Take 125µl of whole blood from the patient sample.
2. Wash 4 times in isotonic saline.
3. Resuspend final cell pellet to 2.0ml in isotonic saline.
4. Prepare duplicate 100µl aliquots of cell suspension.

### Control Preparation

Positive Controls:  
Prepare positive controls with 0.25% and 1% RhD positive cells in RhD negative cells.

Negative Control:  
Prepare negative control using 100% RhD negative cells.

## Test Procedure

1. Into each prepared sample/control tube dispense 50µl of FITC Anti-D with immediate thorough mixing.
2. Incubate tests for 30 minutes (±5 minutes) at 37°C, protected from light.
3. Wash the cells 4 times in isotonic saline to remove unreacted FITC Anti-D conjugate.
4. Resuspend the final cell pellet in 1.5ml filtered isotonic saline or the appropriate medium recommended by the instrument manufacturer.
5. Count 500,000 events for each control and sample.

## INTERPRETATION OF RESULTS

### Analysis

Samples are analysed using forward and side scatter parameters. Gates should be selected such that >99% of red cells are analysed for fluorescent staining. Gain setting on FL1 or FL2 are adjusted such that <5% of negative events fall below the first log decade. Regions must be set on FL1 or FL2 to separate negative and positive cells such that less than 0.05% of negative events fall in the positive region. Appropriate controls prepared from ABO-compatible RhD negative and RhD positive materials should be analysed in parallel with each set of samples:

- 100% RhD negative cells.
- 99% RhD negative and 1% RhD positive (approximating a 22-mL FMH).
- 99.75% RhD negative and 0.25% RhD positive (approximating a 5.5-mL FMH).

It is recommended that duplicate test samples are analysed and that no less than 500,000 events collected. If duplicate tests are within 10% of each other, the mean value should be accepted. If duplicate test gives value outside 10% limits, the test should be repeated. For example, a 10% tolerance range for 500,000 events means 500,000 +/- 25,000 (i.e. 475,000-525,000).

Labelled cell counts are corrected by subtraction of the background value in the gated channel for the RhD negative standard. The volume of fetal bleed for each sample is obtained by calculation as described in the Guidelines<sup>(1)</sup>.

### Calculation of FMH

The volume of the FMH is calculated using the formula described by Mollison (1972)<sup>(2)</sup>, which assumes that the maternal red cell volume is 1800 ml and that the fetal cells are 22% larger than maternal cells.

Calculate percentage RhD positive as:

$$\frac{\text{Sample count} - \text{background count}}{\text{Total count}} \times 100 = \text{A}\%$$

Calculate equivalent fetal bleed as:

$$\text{A}\% \times 1800 = \text{B}$$

Correct for fetal cell volume

$$\text{B} \times 1.22 = \text{Volume of fetal cell haemorrhage}$$

\* Total count = 500,000 red cells.

\*\* Sample count = labelled RhD positive cells within total count.

## QUALITY CONTROL

Quality control of the test should be performed with each set of samples.

Independently constructed controls at 1% RhD positive and 0.25% RhD positive should be run as samples and confirmed to be within +/-5% of the appropriate simulated fetomaternal haemorrhage volumes of 22ml and 5.5ml<sup>(1)</sup>.

Appropriate quality control checks on the operation of the flow cytometer should be performed as recommended by the manufacturer.

## PERFORMANCE LIMITATIONS

FITC Anti-D is not suitable if treatment has been given prior to sample collection. In which case, results should be confirmed by another method<sup>(1)</sup>.

The cell washing procedure must be validated to ensure that all cells are sedimented by the centrifuge and are thoroughly resuspended before staining.

The expression of certain red cell antigens may diminish in strength during storage, particularly in EDTA samples. Better results will be obtained with fresh samples.

False positive or false negative results can occur due to contamination of test materials, improper reaction temperature, improper storage of materials and omission of test reagents.

## SPECIFIC PERFORMANCE CHARACTERISTICS

LDG 76 is a human heterohybrid monoclonal antibody specific for epD3 in the nine epitope model and epD5 in the thirty-six epitope model of the RhD antigen.

This antibody recognises all clinically significant partial RhD antigens<sup>(3)</sup>.

However, where mother and baby have been identified as having a partial RhD antigen type, it may be best to use a test which detects fetal haemoglobin.

When properly stored and used according to the procedures described under 'Recommended Technique' this reagent will give an estimation of fetomaternal haemorrhage volume within the range 0 - 44ml.

This reagent meets the requirements of Directive 98/79/EC on *in vitro* Diagnostic Medical Devices and the recommendations contained in the Guidelines for the Blood Transfusion Services in the United Kingdom.

## REFERENCES

1. BCSH Guidelines: The estimation of fetomaternal haemorrhage. *Transfusion Medicine*, 1999, **9**, 87-92.
2. Clinical Green Top Guidelines- Anti-D immunoglobulin for Rh prophylaxis. [www.rcog.org.uk](http://www.rcog.org.uk)
3. Semi-automated data analysis of flow cytometric estimation of FMH in D- women. *Transfusion*, 2002, **42**, 1067-1078.
4. Mollison, P.L. (1972) Quantitation of transplacental haemorrhage. *British Medical Journal*, **3**, 31-34.
5. Standards for Blood Banks and Transfusion Services AABB, *Advancing Transfusion and Cellular Therapies Worldwide*, 23<sup>rd</sup> Edition, 2004.

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