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# IS $D^U$ PHENOTYPE TESTING A NECESSITY IN BLOOD BANK PRACTICE IN NIGERIA?

**C. A. Nwauche, O. A. Ejele**
*Department of Haematology University of Port Harcourt Teaching Hospital Port Harcourt*

## ABSTRACT

**Objective:** To evaluate the necessity for $D^U$ phenotype testing in blood bank practice in Nigeria.

**Design:** Cross-sectional study

**Setting:** Three health institutions within Port Harcourt metropolis: The University of Port Harcourt Teaching Hospital, Braithewaite Memorial Hospital, Port Harcourt and Orogbum comprehensive health centre, Port Harcourt.

**Sample:** One hundred and fifty Rhesus Negative pregnant woman.

**Method:** Three millilitres (mls) of blood was collected from each subject by venepuncture, into 10mls sterilin containers and the red cells were then separated from the serum before embarking on the two stage $D^U$ testing. (according to Dacie and lewis)

**Results:** 105 Rhesus Negative pregnant females were tested out of which only 1 (0.95%) was $D^U$ phenotype positive while the rest 104 (99.05%) were $D^U$ phenotype Negative (True Rhesus Negative).

**Conclusion:** The pattern of $D^U$ phenotype appears not to be uniform in Nigeria. It is high in Western Nigeria and low in the South Eastern and Niger Delta, while the incidences in Northern Nigeria is yet to be evaluated. Hence, $D^U$ phenotype testing may be necessary, if not mandatory in areas of high incidence while it may not be necessary in areas of low incidence. More studies are required to evaluate the rationale approach to $D^U$ testing in Northern Nigeria.

## INTRODUCTION

The D antigen amongst Rhesus D positive people is known to exhibit considerable variation in the strength of its immunogenicity. Thus, the $D^U$ red cell is said to have fewer D antigen sites per red cell than normal D positive cells, hence they present with weaker – than expected D antigen that may be mistyped as Rhesus D negative[1,2,3]. These Rh D antigen ($D^U$ Phenotype) cells vary quantitatively and qualitatively such that they may make allo-anti-D due to the fact that they lack part of the D mosaic. That is, they carry a partial D antigen and have made antibody to the missing part of the D antigen[4]

There are various grades of the $D^U$ phenotype namely:- The low, intermediate and high-grade $D^U$ phenotype red cells. In routine blood bank practice, the red cells are identified by various methods which include:

(a) Agglutination method using saline anti D and Albumin anti D (detection of high grade $D^U$)
(b) Sensitive enzyme method
(c) Indirect Antiglobulin test
(d) Adsorption and Elusion method. The following tests: b, c, d, above identify the low grade $D^U$ phenotype [5,6]

In the management of Rhesus Negativity, $D^U$ phenotype is critical in order to prevent the grave clinical sequelae of haemolytic disease of the newborn and haemolytic transfusion reaction. The $D^U$ phenotype test also eliminates TRUE Rhesus Negatives from D mosaic / weak D antigen individuals. Thus it helps to prevent the administration of Rhogam to falsely typed Rhesus D Negative pregnant women. It also helps to prevent the administration of the scarce Rhesus Negative blood to $D^U$ individuals mistyped as Rh D Negative and also eliminates the possibility of immunizing a TRUE Rh-negative woman with $D^u$ Red cells has been reported.[6]

In this environment, very little work has been done to properly evaluate the management of Rhesus Negative and $D^u$ phenotype apart from the classic studies of Worlledge et al [7,8,9] which was localized to the western part of Nigeria. Thus, this study was carried out in Port Harcourt to generate data that would afford the reappraisal of the necessity of routine $D^u$ phenotyping and the management of Rh Negativity in Nigeria by screening adult females to identify Rh-negative subjects who were $D^U$ phenotyped through the use of the indirect Antiglobulin test.

*Correspondence: C. A. Nwauche

## SUBJECTS AND METHODS

### Subjects

105 Females within the child- bearing age brackets of 15-45 years from various ethnic groups were randomly screened from three Health Institutions within Port Harcourt. Metropolis:

(a) The University of Port Harcourt Teaching Hospital
(b) Braithewaite Memorial Hospital, Port Harcourt
(c) Comprehensive Health Center Orogbum, Port Harcourt.

### Methods

Three milliliters ( mls) of blood was collected from each subject by venepuncture, into 10mls sterlin containers and the red ells were then separated from the serum before embarking on the two-stage $D^U$ testing (according to Dacie and Lewis, 1991)[5]

### Initial Testing

The Rh grouping of all samples received into the blood banks of the project centers was carried out by the standard tile technique[5], using potent commercially prepared saline anti-D of different batches (Laboratory Diagnostic products and Biotic Laboratories) by mixing one drop of 20% cell suspension to two drops of saline Anti-D sera (cell to serum) on Perspex tile. Negative and positive controls were set up at this stage. All samples that showed agglutination both visually and microscopically were read as being Rh D positive and the procedure was halted while those that showed no agglutination proceeded to step II: $D^U$ testing.

### Indirect Antiglobulin Technique

All samples that were found to be Rhesus Negative were further tested by the Indirect Antiglobulin Technique by incubating the sample/test red cells with the "incomplete" anti-D antisera for 30 minutes at 37°C, washing (3-4 times with normal saline) and their subsequent reaction with potent antihuman globulin: (Coomb's reagent) of different batches (Laboratory Diagnostic products and Biotic Laboratories). Negative and positive controls were also set up.

All samples that showed agglutination both visually and microscopically were read as $D^U$ positive while those that showed no agglutination were read as $D^U$ Negative.

## RESULT:

A total of 1,108 adult within the 15-45 age group were randomly screened. 1003 (90.5%) samples were Rh-positive while 105 (9.5%) samples were Rh-negative. Furthermore, only 1 (0.95%) sample amongst the Rh-negative samples tested as $D^U$ Positive and was of the Ibo ethnic group while 104 (99.05%) were $D^U$ Negative (True Rhesus Negatives). Thus, the prevalence of Rhesus Negativity in Port Harcourt was 9.5% while that of $D^U$ Phenotype amongst the – Negative adult females in Port Harcourt was found to be 0.95%.

Table 1 shows the prevalence of Rh-negative in the various ethnic groups screened to obtain a total of 105 Rh-negative samples. The data on Table 2 on the other hand shows the prevalence of $D^U$ phenotype amongst the Rh-negatives. Here, only (0.95% sample was found to be $D^u$ phenotype and was of the Ibo ethnic group forming (2.13%) of all Ibo Rh-negatives.

*Table 1:* **Prevalence of Rh-negative Amongst Adult Females**

| Ethnicity | Rhesus Negative | Rhesus Positive | Total |
|---|---|---|---|
| Ibo | 47 (4.24%) | 363 (32.76%) | 410 (37%) |
| Ijaw | 40 (3.61%) | 439 (39.62%) | 479 (43.23%) |
| Ikwerre | 4 (0.36%) | 61 (5.51%) | 65 (5.86%) |
| Yoruba | 7 (0.63%) | 35 (3.16%) | 42 (3.79%) |
| Efik-Ibibio | 3 (0.27%) | 66 (5.96%) | 69 (6.22%) |
| Edo | 2 (0.18%) | 5 (0.45%) | 7 (0.63%) |
| Ogoni | - | 16 (1.44%) | 16 (1.44%) |
| Hausa | 2 (0.18%) | 18 (1.63%) | 20 (1.80%) |
| Total | 105 (9.5%) | 1003 (90.5%) | 1108 (100%) |

*Table 2:* **Prevalence of $D^U$ Phenotype amongst Rh-negative Females**

| Ethnicity | Rhesus Negative | Rhesus Positive | Total |
|---|---|---|---|
| Ibo | 47 (44.76%) | 1 (0.95%) | 46 (43.81%) |
| Yoruba | 7 (0.67%) | - | 7 (6.67%) |
| Ikwerre | 4 (3.81%) | - | 4 (3.81%) |
| Ijaw | 40 (30.10%) | - | 40 (30.10%) |
| Edo | 2 (1.90%) | - | 2 (1.90%) |
| Efik-Ibibio | 3 (2.86%) | - | 3 (2.86%) |
| Hausa | 2 (1.90%) | - | 2 (1.90%) |
| Total | 105 (100%) | 1 (0.95%) | 104(99.05%) |

## DISCUSSION:

It has been reported that the incidence of $D^U$ phenotype in caucasians is low, being 0.6% in the UK[10]. The findings in this UK study contradicts the findings in blacks in whom it is reported to be higher[11]. This high trend was reported by David-West[12] who noted that the $D^U$ phenotype frequency among the Yorubas of Western Nigeria was as high as 7.5% in donors who are Rh-negative.

However, this apparent high level of $D^U$ phenotype was not confirmed by the findings in this study in which it was found to be 0.95%, which is similar to the incidence among Caucasians. Thus, it would appear that the distribution of $D^U$ phenotype in Nigeria is not uniform.

The implications of this in terms of $D^U$ phenotyping is that $D^U$ testing is necessary, if not mandatory in areas of high prevalence such as Western Nigeria while it may not be necessary in area of low prevalence such as Port Harcourt (according to this study) and South Eastern Nigeria[7] where Worlledge et all reported that $D^U$ phenotype was not found in their study.

Furthermore, the differential pattern of $D^U$ phenotype prevalence in Nigeria may also suggest that it would be both clinically expedient and cost effective for all adult females in area of high low-grade $D^U$ prevalence to have the $D^U$ test carried out on them before subsequent prophylactic Rhesus therapy in order to identify the true Rh-negative who requires this treatment. This approach would not only prevent the inadvertent immunization of true Rhesus with $D^U$ phenotype red cells from subjects who were mistyped as Rh-negative but would also curtail or prevent the wastage of scarce Rh-negative but would also curtail or prevent the wastage of scarce Rh-negative blood if transfused to $D^U$ phenotype individuals.

## CONCLUSION

The findings of this study show that $D^U$ testing may not be a necessity in all parts of Nigeria. Thus, it may be recommended in Western Nigeria due to the high prevalence of low-grade $D^U$ while it may not be necessary in the Niger Delta area (Port Harcourt and its environs) of Nigeria. In parts were no relevant data exist such as Northern Nigeria, the approach to be adopted is uncertain and calls for further research.

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