Normative Values of Serum Immunoglobulins by Single Radial Immunodiffusion: A Review

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Many previous studies of normative values in adults have suggested that race, age, sex, and environment all have significant effects on the mean values for IgG, IgA, IgM, and IgE in various groups of individuals. Single radial immunodiffusion is the technique most widely used to quantitate immunoglobulins of the three major classes (IgG, IgM, and IgA) in sera. Measurements have been expressed in terms of mass concentration, as percentages of the mean normal adult value, and in arbitrary international units. To improve agreement among laboratories, the WHO has supported the distribution of an International Reference Preparation for the Human Immunoglobulins IgG, IgA, and IgM, and similar (separate) preparations for IgD and IgE.

Additional Keyphrases: factors affecting concentrations • reference materials • techniques • reference values

This survey will largely be directed to a review and discussion of normative values of IgG, IgA, and IgM in human serum from adults, with only brief comments on the findings in children and on the present status of normative values for IgD and IgE. Reviews on the subject include those by Kalff (1), Hobbs (2), McFarlane (3), Bradley (4), and Becker (5).

IgG, IgA, and IgM are quantitated by means of three techniques, all of which are based on the fundamental principles of immunoprecipitation as defined by Heidelberger and Kendall (6). The most commonly used quantitative assay is single radial immunodiffusion, which is performed according to either the original technique of Mancini et al. (7) or its modification described by Fahey and McKelvey (8). Another quantitative immunoprecipitation assay is electroimmunoassay or “rocket electrophoresis,” the method of Laurell (9). The third method is nephelometric immunoassay (10–12).

Because of its simplicity, single radial immunodiffusion is the most frequently used means of quantitating immunoglobulins. Not only is this method used as a research tool by a large number of investigators but agarose-antibody plates and reference sera are obtainable as kits in the U.S.A. from at least nine commercial houses. Quantities as low as 10 mg/liter can be precisely measured, and this degree of sensitivity can be increased 20-fold to about 500 μg/liter by procedures such as DL-3,4-dihydroxyphenylanline (DOPA) treatment (13), which intensify the precipitin ring or 250-fold (to 40 μg/liter) by the radioactive double-antibody method (14).

The results of immunoglobulin quantitation have been expressed in familiar units of mass concentration (g/liter), as percentages of mean values for the normal adult population, or in terms of arbitrary international units (arb. units/liter). In early studies of normative values investigators each used their own standard sera and antisera, leading to great variability between laboratories. In an attempt to standardize immunoglobulin quantitation, a cooperative study by expert laboratories showed that use of individual reagents and standards gave unacceptably wide ranges of immunoglobulin values for six coded serum samples (15). Agreement was considerably improved when a single reference preparation was used by the collaborating laboratories. As a result of these studies, a freeze-dried pool of human serum has been prepared and distributed on a worldwide basis under the aegis of the World Health Organization (WHO) (16–19). This International Reference Preparation of Human Immunoglobulin contains 100 int. units each of
IgG, IgA, and IgM. We have discussed the problem of standardization in greater depth in our accompanying paper (20).

Only comparatively few studies of normative values related to the WHO Reference Preparation have been reported (5, 21–26, 55). Previous studies in adults have most frequently used milligram values. The results of some of these investigations of normative values may contain bias because of the selection of the samples studied. In some instances the sera were drawn largely from hospital personnel. In other studies age, sex, or race were not defined for the sample. Screening for monoclonal proteins in individuals with a high concentration of one or more of the immunoglobulins was not done in all investigations. Nevertheless, these earlier studies indicate that a number of factors—including race, age, and sex—significantly affect the mean values for IgG, IgA, and IgM observed in various groups of individuals tested.

In a given normal subject the individual variation of immunoglobulins over periods of five months appears to be very low (27, 28). In a study (29) in Gambia, the immunoglobulin concentrations observed in individual subjects were relatively stable during a year in spite of infectious disease and climatic conditions. According to Hobbs (2), the individual variation usually remains within ±20% over a period of years.

The question of genetic vs. environmental influence on immunoglobulin concentrations is an interesting one. Striking examples of familial incidence of selective immunoglobulin deficiency, particularly of IgA, suggest a genetic influence. However, a study of monozygotic and dizygotic twins by Rowe et al. (30) showed that the genetic effect is small. The results of family studies of immunoglobulin concentrations in Gambia (31) indicate that genetics plays a greater role in the regulation of IgG and IgE than of IgM. In contrast the genetic influence on IgA and IgD concentrations appeared to be very low or negligible.

The concept that environment and antigenic challenge plays the major roles in stimulating immunoglobulin production and maintaining concentrations of the immunoglobulins is strongly supported by results of experiments with germ-free animals. Such animals have very low concentrations of immunoglobulins, which increase when the animal is exposed to environmental antigens.

In a study of children and adults in the U.S.A., Stiehm and Fudenberg (32) did not observe differences owing to race. Hobbs (2) noted that natives of underdeveloped countries have higher IgG and IgM concentrations than do subjects in Britain. These abnormally high immunoglobulin values may reflect environmental differences such as increased infectious diseases; individuals from such underdeveloped countries showed a decrease in immunoglobulin concentrations to 140% of mean adult value in the U.K. after living in Britain for some years. Hobbs (2) suggests that this residual elevation may reflect the genetic survival value of such subjects in their countries of origin. Caution, however, must be used in interpreting the immunoglobulin concentrations of subjects who move from underdeveloped countries to countries such as Britain. These individuals may also reflect advanced economic opportunity in their country of origin. No report could be found in which immunoglobulin concentrations of such groups or individuals were measured before or soon after arrival in Britain and again some months or years later. Cohen et al. (33) observed that West Africans who had lived in Britain for several years synthesized γ-globulin at almost twice the daily rate observed in healthy Europeans. Mohammed et al. (34) observed significant differences in the IgG, IgA, and IgM values in healthy individuals from a Nigerian village and from the Old City of Zaria in Nigeria. It was noted that the pattern of parasitic infections differs in these two communities.

In a study of American Indians in Surinam (35), the IgG, IgM, and IgA values were significantly higher than those of a control group of Dutch subjects. Mean IgG and IgA values for normal adult Indians in Bombay, India, were found to be significantly higher than reported values for Europeans and English (36). A combination of hygienic and climatic conditions the net result of which was an increased antigenic stimulus was considered as a possible explanation. In contrast to the immunoglobulin pattern observed in India, Yadav and Shah (37) found that IgG, IgA, and IgM values for Malaysians fell within the range reported for Caucasians, and below those observed in West Africans.

In the U.S.A., Buckley and Dorsey (21) observed that the mean IgG and IgA concentrations were higher in black males than in white males, while IgM values in these two groups were not significantly different. Karayalcin et al. (38), in New York, reported that both black males and females had higher values for IgG, IgA, and IgM than did white males and females. These authors did not examine environmental factors and therefore concluded that their results did not necessarily reflect a genetic cause.

Sex appears to be a factor influencing immunoglobulin concentrations, particularly IgM. Rhodes et al. (39) found that women with XXX or XXY chromosomes had higher concentrations of IgM than did normal women (XX chromosomes). Normal women, in turn, had higher IgM values than those for men (XY chromosomes). These findings suggest that IgM concentrations are influenced by genes located on the X chromosomes. This role of the X chromosome is substantiated by the observations of Wood et al. (40) that IgM concentrations in normal females are greater than in women with XO chromosomes. The family studies by Grundbacher (41) showed that the correlation in terms of IgM values was much closer between boys and their mothers than between boys and their fathers. The IgM concentrations in girls were more closely correlated with those of their fathers than...
Fig. 1. Normal immunoglobulin concentrations in relation to age
(From W. Becker 1974, courtesy of the author and Charles C Thomas, publisher). O -- G Male; x -- x Female

with those of their mothers. These observations plus the observed higher mean values of IgM in both black and white females support the hypothesis that in humans the X chromosome carries genes that control IgM concentrations.

Stoop et al. (42), in a study on children, found higher concentrations of both IgG and IgM in girls than in boys; they questioned whether these observations reflected either genetic or other influencing factors. The sex-related difference in IgM concentrations is observed as early as four to five years of age (29, 42). No sex-related difference in IgG or IgM values was reported in two other studies involving children (32, 43). In studies of adults, differences in IgG values between males and females have not usually been observed.

The pattern of adult immunoglobulin concentrations in relation to age is not clear cut. The age groups used in the analysis of various studies have frequently differed, making comparisons difficult. Increased concentrations of IgG and IgA were found to be associated with senility in a study by Haferkamp et al. (44), but not by Hobbs (2). Becker (5) summarizes the effect of age as a continuous increase of IgG and IgA in adults up to senility, whereas highest IgM values, particularly in females, are observed during the 5th decade. Becker’s own results (5) on a total of 960 sera are shown in Figure 1. There is a gradual increase in the immunoglobulins with age. A sharp increase in IgA and IgM is observed in males between the ages of 75 and 85 years but IgG reaches a peak a decade earlier. The highest age of the females in this study was a decade lower than that of the males; it therefore is not known whether females also show an increase in IgA and IgM after the 75th year of age.

In a study of 800 U.S. residents (25), we attempted to establish normal adult values for IgG, IgA, and IgM in terms of int. units. All sera were obtained from the Center for Disease Control Serum Bank. The sera were collected over a 10-year period in five states during epidemiological surveys for specific antibodies after viral outbreaks. The sera were stored at −20 °C and were not thawed more than twice during the investigation. The original design of the study required groups of 50 sera from black and white males and females in each of the following age groups: 20–40 years, 41–60 years, and 61–80 years. After the initial study was completed we decided that more reliable estimates of population variables were needed for IgG and IgM data on white men between 61 and 80 years of age, and for IgA data on white women in this same age group. We therefore tested an additional 100 sera in each of these groups.

Fig. 2. Mean IgG values plus and minus two standard errors of the mean for race, sex, and age groups

Fig. 3. Mean IgA values plus and minus two standard errors of the mean for race, sex, and age groups
The measurements were made according to the original technique of Mancini et al. (7) with goat antisera prepared in our laboratories. A secondary standard was calibrated against the WHO Reference Preparation. Four dilutions of the standard and, in addition, one or two internal control sera were included with the nine unknown sera in each plate.

Figure 2 shows the IgG values plus and minus two standard errors of the mean, which we obtained according to race, age, and sex. For all age groups the mean IgG concentration was higher in blacks than in whites. A more modest elevation of IgA in blacks as compared with whites is seen in Figure 3. In addition, except for white females there was a significant increase in mean IgA with age for both races. In the younger adults (age 20 to 40 years) mean IgM values were markedly higher in females than in males (Figure 4). A decrease of IgM with age was observed particularly in the females of both races; however, this did not occur in white males.

There appears to be general agreement between the int. unit values for the adult white U.S. residents in our study and the int. unit values reported for adults by Cejka et al. (24) for U.S. males of unstated race, by Rowe (22) for European males, by Sinkov et al. (23) for Bulgarian males and females, and by

![Image of graph](image-url)

**Fig. 4.** Mean IgM values (int. units/ml) plus and minus two standard errors of the mean for race, sex, and age groups

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<th>Table 1. Serum Immunoglobulin Concentrations of Healthy Adult U.S. Whites and Europeans</th>
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<td><strong>Mean immunoglobulin concn, int. units/ml</strong></td>
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<td><strong>Investigator</strong></td>
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<td>Maddison et al. 1976</td>
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* Empirical 5th and 95th percentiles

* Range

**Note:** To convert to milligrams per liter, multiply these values by 80.4 for IgG, 14.2 for IgA, and 8.47 for IgM (See reference 19).

<table>
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<th>Table 2. Serum Immunoglobulin Concentrations of Healthy Young Adult Black Males in the U.S. and Nigeria</th>
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* Empirical 5th and 95th percentiles

* 95% confidence limits
Becker (5) for European males and females. These data are summarized in Table 1. The only group of blacks with which comparison of any of our data appeared possible was the group of Nigerian males in Rowe's report (22). These are shown in Table 2. Because of environmental differences, it was not surprising to find that the IgG and IgM concentrations were higher in the Nigerians than in the U.S. black residents. The low IgA values in the Nigerians is not so readily explained. However, Turner and Voller (45), in a study done in Nigeria, observed IgA values similar to those for persons living in the U.K.: the IgG and IgM values for the Nigerians were significantly higher than those for the British. Shulman et al. (26) did not show data on each of the three immunoglobulin classes studied in respect to the age groups of their South African subjects. However, they also observed higher values in blacks than in whites; in addition, they noted influences of age and sex on immunoglobulin concentrations.

The results of our study show that one cannot conclude that the mean IgG concentrations are affected by age if sex is ignored, or by sex if age is ignored. Except for white women between the ages of 61 and 80 years, there was a significant increase in mean IgA with age for both races. This exception indicates that there is a significant interaction between race and age in relation to IgA values in white women. Lamy et al. (46) observed increased values for IgA in black males and females with age and that IgA values were significantly higher in men than in women between the ages of 45 and 65 years. Except in white males, we observed a marked decrease in IgM levels with age in all subjects, particularly females of both races. A less pronounced decrease occurred in black males. While our results do not parallel those of Cassidy et al. (47) in that we observed decreased concentrations of IgM with age in all groups except white males, and of IgA in white females, we concur with these investigators in our observation of statistically significant interactions between race, age, and sex factors for all three immunoglobulin classes. We suggest that a much larger population study is needed to provide true normative limits.

The apparent effect of seasonal change on immunoglobulin values was observed by McFarlane (48) but not by McGregor et al. (29) in West Africa where the wet season parallels an increased rate of transmission of parasitic and other infectious diseases and, therefore, increased antigenic stimulus. The data reported by Rowe (22) show that healthy young adult males in Mexico City have lower mean IgA and IgM concentrations than do similar groups in most other geographic areas. In the same study, IgG and IgM concentrations in natives of Algiers (Algeria) and Perth (Australia) were shown to be almost identical, but were higher than those in most other areas. However, the IgA values for Perth are considerably lower than those for Algiers. Such factors as altitude, mass vaccinations, and protein intake may constitute persistent environmental differences that affect the immunoglobulin concentrations.

The question of immunoglobulin levels in pregnancy also requires consideration. Cohen et al. (33) found that considerably less γ-globulin was synthesized by a woman who was three months pregnant at the time of testing than was synthesized by the comparable general population in the study. McGregor et al. (29) found mean IgG and IgA values to be significantly lower during pregnancy. The same authors did a longitudinal study on a cross-sectional group; IgG appeared to decrease progressively throughout pregnancy, and reached the lowest values in the last 10 weeks of gestation. IgA also decreased in the first and second trimester, but increased in the third trimester. Maroulis et al. (49) also observed significant decrease of IgG during the second and third trimester and postpartum. Lower IgA values were not observed until postpartum.

Immunoglobulin concentrations of the mother may markedly affect the subsequent values for the neonate (2). Normal full-term infants have IgG concentrations that are nearly the same as those of their respective mothers; such infants have low IgM concentrations and undetectable or only trace amounts of IgA. The upper limit of normality for IgM is usually considered to be 200 mg/liter, but Yeager (50) observed that the cord-blood IgM value varies with birth weight. She suggests that in high-weight babies normal values may exceed 200 mg/liter. However, no such correlation between high weight and IgM values was observed by Winsten (51), nor did Logie et al. (52) find a consistent relationship between IgG and birth weight although they observed a low mean IgG value in infants with lowest birth weight. Premature infants with prolonged gestational periods have detectable amounts of IgA but their IgM concentrations are not abnormally high (53, 54). Some investigators observed slightly supranormal values for IgG in postmature infants (53); others found that prolonged gestation resulted in decreased concentrations of IgG in maternal-blood and cord-blood (54).

Up to 4% of full-term infants fail to replace the maternal IgG in time and may suffer from agammaglobulinemia (2). This may be, but is not usually, due to the mother having subnormal concentrations of IgG. In rare instances the cause has been defined as maternal antibodies reacting against the infant's allelopyotype of IgG, or as maternal paraprotein influencing the infant's synthesis and catabolism of IgG. In premature infants the amount of placentaly transmitted maternal IgG is considerably less than in full-term infants (55). Most of the infants who fail to replace the maternal IgG on time, however, appear merely to be "slow starters" (2), and catch up within a year; serum IgM and IgA concentrations are normal.

It is important that these factors just discussed be taken into consideration in the selection of cord bloods and sera from infants up to one year of age for studies of normative immunoglobulin values.
Studies of immunoglobulin concentrations in children have resulted in some discrepancies (24). Present knowledge suggests that adult values of IgG are reached between six and 10 years of age (24, 42, 43, 56). Some studies suggest that adult IgM values are attained between one and two years of age (27, 43, 56); others suggest they are not reached until about 16 years of age (24, 38). The numbers of samples investigated between birth and age 16 years have usually been small, and various results related to age and sex have been reported. Because of this and because of the probable interrelationships of these and other factors in adults, we suggest that further studies on adequate samples of different populations of children are needed.

A WHO Research Standard for Human Serum IgD is available (57), but few reports of normative values of IgD appear in the literature. Probably the small number of such studies reflects the fact that the functional activity of this immunoglobulin has not yet been defined. Hobbs (2) states that his measurements of IgD in over 2000 sera have provided no clinically useful information. IgD is not usually detectable in cord serum (58, 59), but wide ranges are demonstrable in children and adults. The recent observations of IgD on the surface of peripheral blood lymphocytes of the human newborn (60) and its possible role as a lymphocyte receptor (61) have rekindled interest in this immunoglobulin.

The IgE immunoglobulins are the reaginic antibodies which give immediate hypersensitivity and manifestations of allergy (62). The low concentrations of IgE in serum necessitate the use of quantitative technique of greater sensitivity than simple single radial immunodiffusion. By intensifying the precipitate ring with DOPA (13), Sieber and Becker (63) were able to use the single radial immunodiffusion technique in IgE determinations. Both radioactive radial immunodiffusion and competitive protein binding radioimmunoassays have been used by several investigators.

WHO has made available a Research Standard for Human Serum IgE (64) to which international units have been assigned. When the Reference Preparation was used in a collaborative study by five laboratories (65), fairly good agreement between participant results was obtained irrespective of the techniques and antisera used. However, a number of investigators have encountered problems in attempting to compare measurement, particularly of low concentrations of IgE, by different techniques (66).

When one attempts to compare normative IgE values reported in several studies, it becomes obvious that, to date, not only have most investigators used their own individual reference preparations, but also there are a great many modifications of the basic technique. Nevertheless, some basic information on IgE levels is now available. Although maternal IgE is said not to cross the placenta, low concentrations of IgE were demonstrable in all of 37 cord sera examined by Johansson (67). His study indicated that adult values for IgE are reached before seven years of age. A wide range of values was found in healthy adults and children (67–71). In a study of 4700 blood donors, Orren and Dowdle (72) observed a significant decrease in IgE concentrations with age, and females had significantly lower levels of IgE than males. These investigators (73) also showed that IgE values varied in three ethnic groups in South Africa. Higher values were observed in black Africans, intermediate in “coloreds,” and lowest in whites. In studies of Swedish and Ethiopian children (74) and of adults (75) no sex-related differences were found. The higher values for IgE in Nigerian blood donors compared with those in expatriates living in Nigeria and with Swiss blood donors probably reflect the high prevalence of parasitic infections (other than malaria) in Africa, rather than racial differences (76). The results of a family study in Canada (71) suggest that low IgE concentrations are controlled by two dominant genes and that high values occur if one of these genes is absent.

The use of single radial immunodiffusion for quantitating immunoglobulins has been extended to the subclasses of IgG (77, 78). As yet, close agreement has not been obtained by investigators. However, in this new application of the technique the difficulties associated with obtaining representative anti-subclass antisera have been well documented (79). Nevertheless, van der Giessen et al. (77) observed that IgG2 increased slowly with age, with adult values not yet reached at the age of 12 years. IgG4 shows a similar rise but in a lesser degree. In contrast, the IgG1 and IgG3 values of children did not vary significantly from those of adults.

In conclusion, it becomes apparent from this brief review that the effects of environment, race, sex, and age and their interactions have not been fully defined for any of the five classes of immunoglobulins. It is anticipated that the true normal ranges of the various immunoglobulins will vary significantly in subpopulations of man. It is essential that these normal ranges be accurately established if the patient’s immunoglobulin data are to be of the greatest possible diagnostic aid to the clinician.

The question of sample selection for further studies of immunoglobulin concentrations in normal individuals is extremely important. Because of the interaction of age, sex, and environment, Kalff (7) challenges the concept of “normal” human immunoglobulin values, and suggests that in a given individual the observed values can only be interpreted in terms of the values for a control group matched for age, sex, race, and changing and persistent environmental factors.

We suggest that in the U.S.A., a large, truly randomized, and fully documented probability sample of the nation’s population, somewhat similar to the Health and Nutrition Examination Survey (HANES)
References

51. Winston, S., Personal communication.