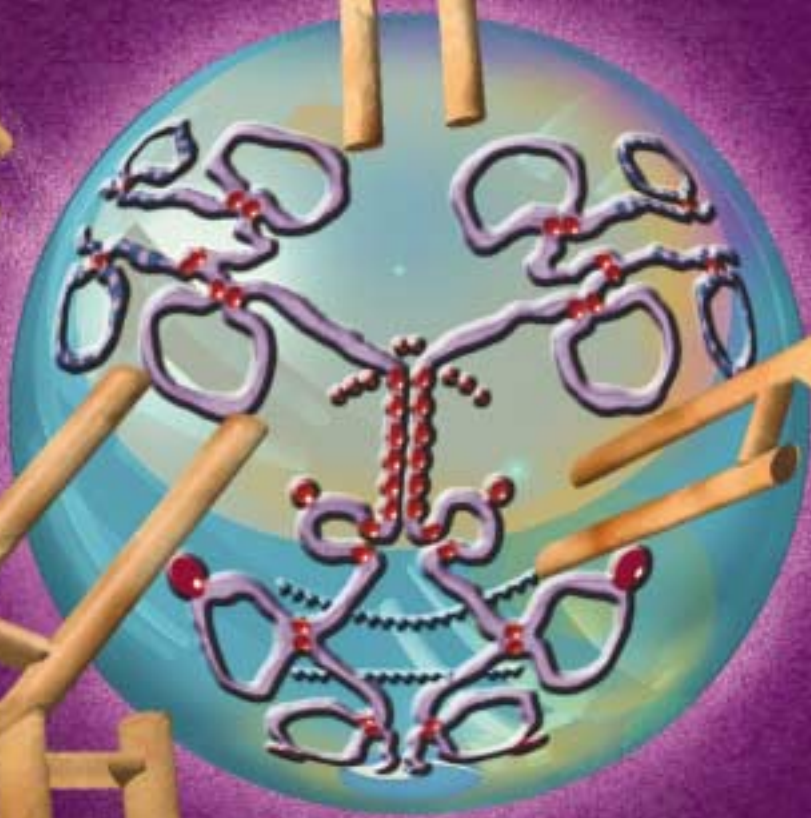




# The Human IgG Subclass



# The Human IgG Subclasses

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*Revised and Edited*

*by*

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We are pleased to present you with this new edition of The Human IgG Subclasses Booklet. As part of our continuing commitment to provide useful product information and exceptional service to our customers, we have compiled this practical resource for investigators who are interested in the rapidly expanding field of quantitation of human immunoglobulins, especially the IgG subclass proteins. Whether you are just beginning your research or are training new researchers in your laboratory, you will find this booklet to be a highly useful reference source.

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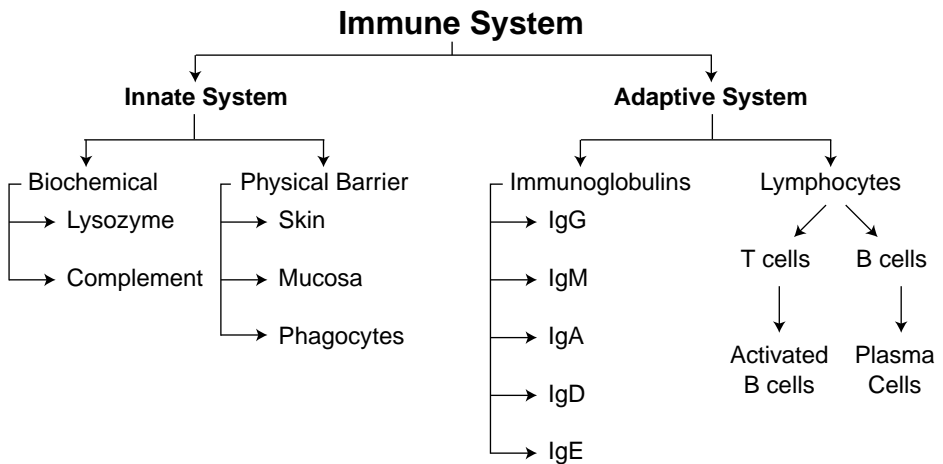
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# I. Introduction

The vertebrate immune system consists of well diversified molecules that recognize and respond to parasitic invasion in a very complex manner (1). The immune system is classified as innate – consisting of barriers to prevent penetration and spread of infectious agents, and adaptive system – consisting of lymphocytes and immunoglobulins. Lymphocytes consist of T cells and B cells that regulate immune response and impart cellular and humoral immunity to the organism. The B cells develop into plasma cells that secrete antibodies. The T cells develop into effector cells that kill infected cells as well as activate macrophages and B cells.



**Figure 1:** Organization of the Vertebrate Immune System

The human immunoglobulins are a group of structurally and functionally similar glycoproteins that confer humoral immunity in humans (2). They are composed of 82-96% protein and 4-18% carbohydrate. The immunoglobulin protein “backbone” consists of two identical “heavy” and two identical “light” chains. Five classes of immunoglobulins (IgG, IgA, IgM, IgD, and IgE) have been distinguished on the basis of non-cross-reacting antigenic determinants in regions of highly conserved amino acid sequences in the constant regions of their heavy chains (3). Four distinct heavy chain subgroups of human IgG were first demonstrated in the 1960’s by using polyclonal antisera prepared in animals immunized with human myeloma proteins (4-6). A World Health Organization (WHO) panel defined them as subclasses 1, 2, 3, and 4 of human IgG based on their relative concentration in normal serum and their frequency of occurrence as myeloma proteins (Table 1) (7). The structure and function of each human IgG subclass protein has been studied extensively, initially with polyclonal antisera rendered monospecific by immunoabsorption and more recently with monoclonal antibodies.

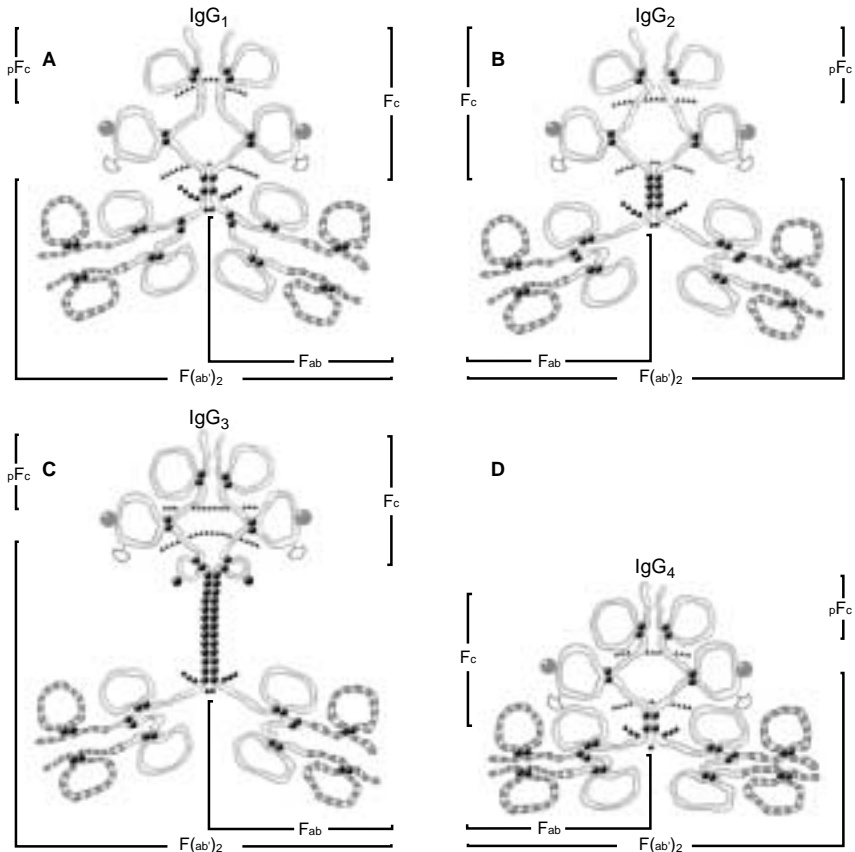
The polyclonal reagents used in IgG subclass studies have not been widely available, and they are difficult to prepare, invariably weak, and frequently contain a heterogeneous mixture of antibodies specific for immunoglobulin subclass-associated allotypes (8, 9). In the 1980's, murine hybridoma technology was used successfully by several groups to produce monoclonal antibodies specific for the human IgG subclass proteins (8, 10-12). The Human Immunoglobulin Subcommittee of the International Union of Immunological Societies (IUIS), supported by the WHO, conducted an extensive collaborative study of 59 monoclonal antibodies with reported subclass specificity by using a variety of immunological assays (13, 14). Highly specific monoclonal antibodies are now available as research and clinical reagents to facilitate quantitation of the level of each IgG subclass in human serum. These antibodies also are being applied to the study of IgG subclass antibodies produced in human immune responses.

This monograph has been prepared as a general guide for investigators who are interested in the rapidly expanding field of quantitation of human IgG subclass proteins. The HP-series of immunochemicals discussed in this monograph includes monoclonal antibodies specific for human IgG PAN, IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, IgG<sub>4</sub>, and the human  $\kappa$  (kappa) and  $\lambda$  (lambda) light chains. This guide is intended only as a summary of basic information and not as an all-inclusive compendium of facts regarding the human IgG subclasses. First, physical, chemical, and biological properties of the human IgG subclasses are summarized. Second, methods are discussed that are used in the preparation, isolation, and quality control of the HP-series monoclonal antibodies. Third, applications for these monoclonal antibodies are examined, with emphasis on measurement of the level of IgG subclasses 1, 2, 3 and 4 in human serum and detection of IgG subclass antibodies by immunoassay. Finally, a bibliography is provided that directs the reader to past research and current trends in the study of human IgG subclasses in human health and disease.

# II. Properties of the Human IgG Subclasses

## Physical and Chemical Properties

The human IgG subclasses are glycoproteins (approx. 150 kDa) composed of two heavy (2 x 50 kDa) and two light (2 x 25 kDa) chains linked together by interchain disulfide bonds (15-17). Intra-chain disulfide bonds are responsible for the formation of loops, leading to the compact, domain-like structure of the molecule. Schematic diagrams of IgG 1, 2, 3 and 4 are presented in Figure 2. There are two types of light chains, which are referred to as lambda ( $\lambda$ ) and kappa ( $\kappa$ ) chains. The ratio of  $\kappa$  to  $\lambda$  varies from species to species, (e.g., in



**Figure 2.** Schematic diagram of the four subclasses of human IgG. The figure shows the major pepsin cleavage points (▲▲▲), major papain cleavage points (●), C1q binding site exposed (☺), C1q binding site exposed only in isolated Fc fragments (☹), constant region of heavy and light chains (≡), variable region of the heavy and light chains that contribute to the antigen binding site (≡) and the carbohydrate side chains (●). Reproduced with permission from *Immunology Today*, June 1980.



mice 20:1, in humans 2:1). This ratio can sometimes be used as a marker of immune abnormalities.

The amino terminal regions of the heavy and light chains exhibits highly variable amino acid composition (referred as  $V_H$  and  $V_L$  respectively). This variable region is involved in antigen binding. In contrast to the variable region, the constant domains of light and heavy chains are referred as  $C_L$  and  $C_H$  respectively. The constant regions are involved in complement binding, placental passage, and binding to cell membrane. Differences in the amino acid content of the heavy chains and the ratio of  $\kappa$  to  $\lambda$  light chains are characteristic of the different subclasses of IgG. While the primary amino acid sequences of the constant regions of the IgG subclass heavy chains are greater than 95% homologous, major structural differences are found in the hinge region in terms of the number of residues and interchain disulfide bonds (Table 1).

The hinge region is the most diverse structural feature of different IgGs. It links the two Fab arms to the Fc portion of the IgG molecule and provides flexibility to the IgG molecule. Also, it forms a connecting structure between the two heavy chains. The flexibility of the hinge region is important for the Fab arm to interact with differently spaced epitopes, and for the Fc region to adapt different conformations. The disulfide bonds in the middle hinge region are important for covalent linking of the heavy chains.

The IgG<sub>1</sub> hinge is 15 amino acid residues long and is freely flexible so that the immunoglobulin regions or fragments that bind antigen (Fabs) can rotate about their axes of symmetry and move within a sphere centered at the first of two interchains disulfide bridges (18). IgG<sub>2</sub> has a shorter hinge than IgG<sub>1</sub>, with 12 amino acid residues and four disulfide bridges at the Fab base. The hinge region of IgG<sub>2</sub> also lacks a glycine residue, which together with its shortness almost completely prevents rotation and restricts lateral movement of the Fabs (19). IgG<sub>3</sub> has a unique elongated hinge region containing 62 amino acids (21 prolines and 11 cysteines) that has been described as an inflexible polyproline double helix (16, 19-21). The IgG<sub>3</sub> Fabs appear to rotate and wave at a rate similar to those in IgG<sub>1</sub>; however, remoteness of the Fc (crystallizable fragment) from the Fab causes the Fab to be less frequently near the Fc over time. This makes it more readily available for binding of complement component 1q (C1q) to the Fc region of IgG<sub>3</sub> in solution in comparison with its binding to IgG<sub>1</sub> Fc. Finally, IgG<sub>4</sub>'s hinge is shorter than that of IgG<sub>1</sub>, its flexibility is intermediate between IgG<sub>1</sub> and IgG<sub>2</sub> and some rotation may occur around the glycine residue in its hinge region. Access of C1q to the IgG<sub>4</sub> Fc is hindered by the shortness of the IgG<sub>4</sub> hinge, which leads to the Fabs spending more time close to the Fc (19).

The point of light chain attachment to the heavy chain also differs among the subclasses. IgG<sub>1</sub> light chains are bound near the midpoint of the heavy chain, while those of IgG<sub>2</sub>, IgG<sub>3</sub> and IgG<sub>4</sub> are joined one quarter the distance from the heavy chain amino termini (19) (Figure 1). Intrachain disulfide bonds of the heavy and light chains transform parts of the molecule into compact globular regions called domains. These domains participate in the biological functions of the immunoglobulin. Unique antigenic determinants are generally found in

the Fc region of IgG<sub>1</sub> and IgG<sub>2</sub>, the hinge region of IgG<sub>3</sub> and the Fc and Fd regions of IgG<sub>4</sub> (22).

Genetic markers (Gm allotypes) are regular minor differences in primary amino acid sequences between molecules of one IgG subclass that occur throughout a species as a result of gene mutation (23-26). In humans, some allotypic markers are restricted to constant region domains of single IgG subclasses, while others are shared by several subclasses. Examples of shared or isoallotypes are Gm<sub>4a</sub>, which has been detected on some human IgG<sub>1</sub>, IgG<sub>3</sub> and IgG<sub>4</sub> molecules, and Gm<sub>4b</sub>, which is shared by human IgG<sub>2</sub> and IgG<sub>4</sub> molecules (27). In humans, certain allotypes have been associated with increased and decreased antibody responses to a variety of bacterial pathogens, autoantigens, isoantigens, tumor antigens, and dietary antigens (28). Excellent discussions of the human IgG allotypes and their importance are presented elsewhere (23, 24, 28-42).

The sedimentation coefficient of the four IgG subclasses is the same ( $S = 7$ ). Early studies indicated that isoelectric focusing (IEF) may be useful in the separation of the four human IgG subclasses based on differences in their net charges (43-45). More recent studies using two-dimensional gel electrophoresis of serum from patients with monoclonal gammopathies have shown that the IgG subclasses are not separated readily by charge alone because their pI ranges overlap each other between pH 6.4 and 9.0 (46) (Table 1). Characterization of the human IgG subclasses has been accomplished in part by digesting IgG subclass preparations with proteolytic enzymes such as papain (47, 48), plasmin (49), trypsin (50), and pepsin (51). Papain, in the presence of cysteine, digests IgG into two Fab fragments, a Fc fragment, and degradation products. Up to two-hour incubation, IgG<sub>2</sub> protein appears to be resistant to degradation (10-20% digested) with papain, while proteins of the other subclasses are completely degraded (52). Pepsin digests IgG into F(ab')<sub>2</sub> with intact antigen-binding activity and a pFc' or small polypeptide chains without antibody activity. While IgG<sub>3</sub> and IgG<sub>4</sub> appear to be relatively more sensitive to pepsin digestion, all four subclasses can be digested eventually. Studies of structure and function of the proteins of the human IgG subclasses by using enzymatically digested fragments are reviewed elsewhere (16, 53).

## Biological Properties

The biological properties of the human IgG subclasses may be categorized as specific reactions of the Fab region with antigen (primary function) and effector (secondary) functions. These reactions occur as a result of antigen binding and are mediated through interaction of the constant regions of the heavy chain, especially the Fc. Principal secondary biological functions of the four human IgG subclasses are summarized in Table 2.

The concentration of each immunoglobulin in serum of healthy individuals depends in part on the number of plasma cells that produce that particular immunoglobulin, the rate of synthesis, catabolism, and the exchange between intra- and extravascular spaces. Adults have the highest concentration of IgG<sub>1</sub> (5-

12 mg/ml), followed by IgG<sub>2</sub> (2-6 mg/ml), IgA<sub>1</sub> (0.5-2 mg/ml), IgM (0.5-1.5 mg/ml), IgG<sub>3</sub> (0.5-1.0 mg/ml), IgG<sub>4</sub> (0.2-1.0 mg/ml), IgA<sub>2</sub> (0-0.2 mg/ml), IgD (0-0.4 mg/ml) and IgE (0-0.002 mg/ml) (53, 54). The rare IgG subclasses tend to vary more considerably between individuals (2, 15). The IgG concentration of a given individual appears to be related to the Gm allotype which indicates that genetic factors are one variable that determine the overall IgG subclass concentration in serum (28, 37, 55). IgG is detected rarely in secretions (2). The 5:1 ratio of IgG to IgA in serum contrasts with the 1:20 ratio detected in saliva and other secretions. Total IgG is about 100 times lower in cerebrospinal fluid (CSF) than in serum (0.8–7.5 mg/dl), which represents about 12% of the CSF protein (2).

IgG exhibits highest synthetic rate and longest biological half-life of any immunoglobulin in serum. Studies of clearance rates of radiolabeled IgG myeloma proteins *in vivo* have demonstrated a higher catabolic rate for human IgG<sub>3</sub> than for IgG<sub>1</sub>, IgG<sub>2</sub>, and IgG<sub>4</sub> (Table 2) (56). Proteins of all four IgG subclasses can pass from the mother to the fetus through the placenta (57-59). The transfer of IgG antibodies from mother to the fetus appears to be mediated by an active transport mechanism that involves Fc receptors at the syncytiotrophoblast membrane that bind the IgG molecules (60). Differential *in vitro* binding affinity to placental homogenates (IgG<sub>1</sub> = IgG<sub>3</sub>>IgG<sub>4</sub>>IgG<sub>2</sub>) suggests that the transfer of IgG across the placenta may be a selective process (57, 61); however, this theory has not been supported by all studies (62, 63). Factors involved in the development of serum IgG subclass levels from the prenatal through adolescent years are reviewed elsewhere (58, 62, 64-67).

Complement activation is possibly the most important biological function of IgG. Activation of the complement cascade by the classical pathway is initiated by binding of C1 to sites on the Fc portion of human IgG. IgG subclass activation of complement by the alternate pathway has not been demonstrated. The globular heads of C1q interact with amino acids 285–292 or 317–340 in the second heavy chain constant region (C<sub>H</sub>2). Reactivity of complement with IgGs of the four human subclasses varies as a function of steric interference by the Fab arms in the approach of C1q to the C<sub>H</sub>2 sites (IgG<sub>3</sub>>IgG<sub>1</sub>>IgG<sub>2</sub>>IgG<sub>4</sub>) (68, 69). Binding of C1 to IgG<sub>3</sub> myeloma is about 40 times greater than binding to IgG<sub>2</sub>, and binding to IgG<sub>4</sub> generally is not demonstrable. IgG<sub>4</sub> antibodies in fact appear to inhibit immune precipitation and binding of C1q to IgG<sub>1</sub> in complexes containing mixtures of IgG<sub>1</sub> and IgG<sub>4</sub> (70). IgG<sub>4</sub> thus may be considered protective against the biological effects of the complement-fixing antibodies (71).

Another vital function of human IgG is its ability to bind to cell surface Fc receptors. Once it is fixed to the surface of certain cell types, the IgG antibody can complex antigen and facilitate clearance of antigens or immune-complexes by phagocytosis. Three classes of human IgG Fc receptors (FcR) on leukocytes have been reported: the FcR-I, FcR-II, and low-affinity receptor [FcR-Io] (72). These are distinguished by their presence on different cell types, by their molecular weights and by their differential abilities to bind untreated or aggregated IgG myeloma protein of the four subclasses. Molecular weights of the IgG Fc receptor molecules are reportedly 72 kDa (FcR-I), 40 kDa (FcR-II) and 50–70 kDa (FcR-Io). The receptors are expressed differentially on overlapping

populations of leukocytes: FcR-I on monocytes; FcR-II on monocytes, neutrophils, eosinophils, platelets, and B cells; and FcR-Io on neutrophils, eosinophils, macrophages, and killer T cells (72).

FcR-I reportedly possesses greater affinity for IgG<sub>1</sub> and IgG<sub>3</sub> ( $K_a = 10^8$  to  $10^9$  M<sup>-1</sup>) than for IgG<sub>2</sub> or IgG<sub>4</sub>. IgG<sub>4</sub> binds less effectively, and IgG<sub>2</sub> proteins almost never bind to FcR-I. Estimated cell surface density of FcR-I receptors on monocytes is  $1 - 4 \times 10^4$  per cell. Studies of the FcR-I specificity compare well with earlier reports that monocytes have Fc receptors preponderantly for IgG<sub>1</sub> and IgG<sub>3</sub> (73-75). FcR-II specificity has been evaluated only on platelets. Aggregated human IgG myeloma proteins of all four subclasses are able to release <sup>3</sup>H-serotonin from platelets, indicating the presence of receptors for all subclasses on the human platelet (76). Use of oligoclonal IgG has shown that platelets bind IgG<sub>1</sub> = IgG<sub>3</sub> > IgG<sub>2</sub> and IgG<sub>4</sub>. Addition of complement to the medium inhibits the release of serotonin from platelets incubated with aggregated IgG<sub>1</sub> and IgG<sub>3</sub>, but not with IgG<sub>2</sub> and IgG<sub>4</sub>. This suggests that complement binds to IgG aggregates and sterically hinders the reaction of IgG Fc with the platelet receptor (2). The low-affinity human IgG receptor has not been well defined. Studies of the neutrophil have shown preferential binding of IgG<sub>1</sub> and IgG<sub>3</sub> to FcR-Io. Release of lysosomal enzymes such as  $\beta$ -glucuronidase from neutrophils by incubation with aggregated IgG myeloma proteins indicates that all subclasses of human IgG can react with the neutrophil (75, 77, 78). Study of IgG subclass Fc receptors on human lymphocytes by using human myeloma proteins has demonstrated that IgG<sub>1</sub>, IgG<sub>2</sub> and IgG<sub>3</sub> can bind to lymphocytes and inhibit lymphocyte cytotoxicity (79).

Human IgG subclasses are known to bind to other proteins. The Fc region of human IgG<sub>1</sub>, IgG<sub>2</sub> and IgG<sub>4</sub> binds to protein A from *Staphylococcus aureus* (80, 81). A single substitution of arginine for histidine at amino acid 435 in the Fc region prevents binding of protein A to IgG<sub>3</sub> (82). Patients with cystic fibrosis can express a factor in their serum that is a heat- and acid-labile low-molecular-weight protein that binds to the constant regions of human IgG<sub>1</sub> and IgG<sub>2</sub> (83). Human rheumatoid factors (RF) are IgG, IgA or IgM antibodies that bind to the Fc of immunoglobulins (84). In most cases, IgG is also the antigen for RF. Human rheumatoid factors react most strongly with IgG<sub>1</sub> myeloma proteins followed by IgG<sub>2</sub> and IgG<sub>4</sub>. IgG<sub>3</sub> appears to be unreactive with RF (85). The biological significance of differential binding of the human IgG subclasses to human leukocytes and human or foreign proteins is discussed in detail elsewhere (2, 53, 86).

This overview has summarized major differences in the structure and effector functions of the four human IgG subclasses. At present, the precise role of each IgG subclass protein within the totality of the immune response remains to be elucidated. The observation that seemingly healthy individuals may be deficient in one IgG subclass challenges the notion the IgG subclass proteins have unique and essential roles in an immune response. However, certain antigenic challenges (e.g., bacterial and viral antigens, allergens) elicit a selective increase in IgG antibodies of certain subclasses (71, 87, 88). Thus, as has been postulated, emergence of the IgG subclasses may permit the efficiency of certain effector functions to be optimized within individual subclasses (16).

# III. Human IgG Subclass-Specific Monoclonal Antibodies

## Preparation

The HP-series of human IgG specific monoclonal antibodies was produced from documented hybridoma cell lines that were developed at the Centers for Disease Control in Atlanta, Georgia, U.S.A. (89). Hybridomas were maintained in cell culture in RPMI with penicillin-streptomycin-fungizone and 10% fetal calf serum for 2 – 8 weeks before use. Antibody-containing ascites (in lots of 500–1500 ml) was prepared by injecting hybridoma cells ( $2 - 5 \times 10^6$  viable cells per mouse) intraperitoneally into 80 to 100-day-old BALB/c mice that had been primed 2 weeks earlier with 0.5 – 1 ml of pristane. The ascites was harvested 5 – 10 days after the injection of cells and immediately centrifuged to remove erythrocytes, lipid, and pristane. Filtered ascites was frozen at  $-70^\circ\text{C}$  without azide. The clone number, murine isotype, and pI of the HP-series on monoclonal antibodies are presented in Table 3.

## Isolation

Monoclonal antibody was purified chromatographically from ascites for covalent coupling to affinity chromatography matrices; adsorption to immunoassay solid phases; conjugation with biotin, enzymes, or fluorescent molecules; or labeling with radioiodine. Routine isolation was performed by using DEAE ion exchange chromatography (90) followed by hydroxylapatite (Cat. No. 391947 and 391948) chromatography (91). Protein A affinity purification was avoided to eliminate any possibility of contaminating the purified monoclonal antibody with protein A, itself a human IgG binding protein (see literature survey.)

Ascites was dialyzed (15,000 M.W. exclusion) overnight against 0.05 M Tris, pH 7.7 at  $+4^\circ\text{C}$ , applied to a column containing DEAE cellulose and eluted with a step gradient by using 0.05 M Tris containing 0 to 0.15 M NaCl. Protein peaks were monitored by absorbance at 280 nm and fractions around protein peaks were analyzed by ELISA (92) to identify immunoreactive monoclonal antibody. Column fractions containing antibody were concentrated two- to ten-fold (Amicon, YM10 membrane) and analyzed by ELISA, isoelectric focusing (IEF), and/or immunoelectrophoresis (IEP) (92, 93).

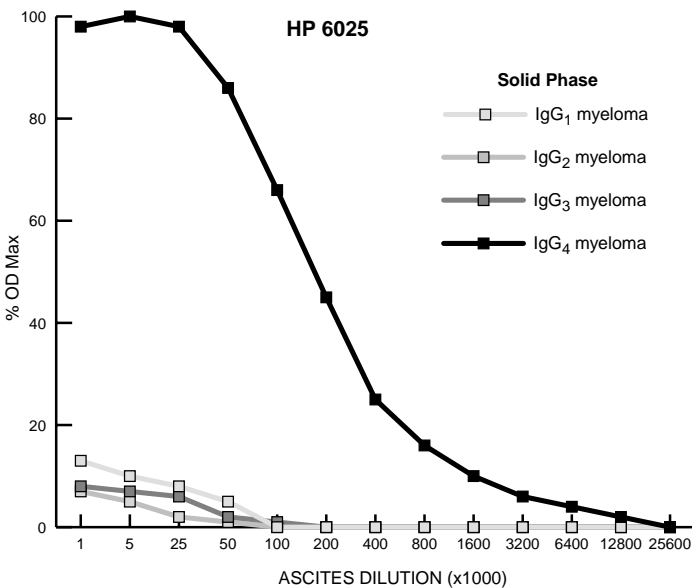
DEAE-isolated monoclonal antibody that contained any detectable contaminants was subjected to hydroxylapatite chromatography (91). Antibody was dialyzed in 0.01 M sodium phosphate buffer, applied to the hydroxylapatite column and eluted with 0.01 M sodium phosphate buffer followed by stepwise increase in sodium phosphate concentration. The actual salt gradient was designed around the known pI of the monoclonal antibody. Analysis by ELISA, IEF and/or IEP was repeated, and column fractions containing purified monoclonal antibody were pooled, concentrated to 2 – 5 mg/ml (based on optical density), aliquoted and frozen at  $-20^\circ\text{C}$ . Protein content of the IgG was determined by  $A_{280}$  ( $E^{1\%}_{1\text{cm}} = 15$ ) and by protein assay by using purified mouse IgG standards.

# Analysis and Quality Control

Laboratory analysis of each lot of monoclonal antibody was performed in three stages from production to final product. First, the cell culture medium was analyzed for the presence and relative amount of human IgG-specific antibody by ELISA before hybridoma cells were injected into mice. Second, collected ascites was analyzed for potency and antibody specificity by using dilutional analysis in ELISA and by IEF, often in combination with an affinity immunoblot (92, 93). Final purity, quantity, immunoreactivity, and specificity of isolated antibody were documented by using IEF and ELISA.

## Specificity

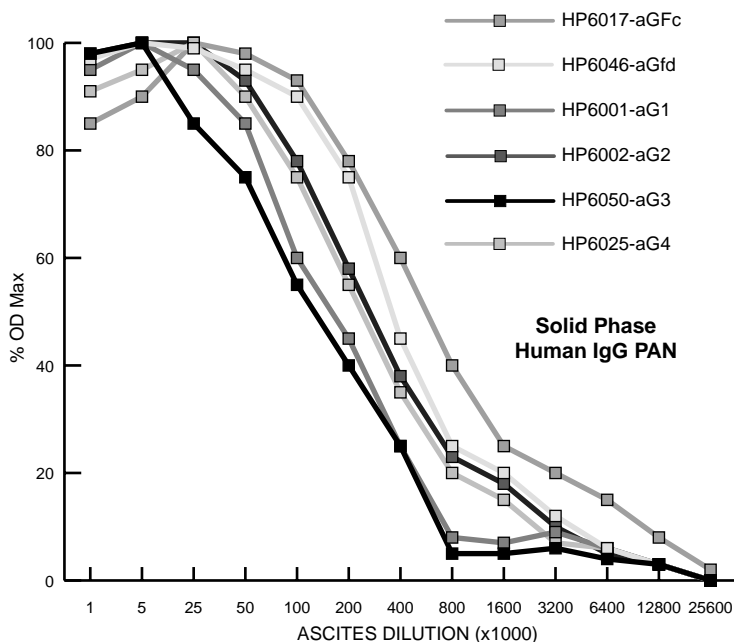
Specificity of each lot of antibody was tested and compared to previous lots and previous reports of specificity for that clone (13, 89, 92, 94). Serial dilutions of ascites samples were analyzed by ELISA by using microtiter plate wells coated with human IgG myeloma proteins of the four subclasses. The ratio of reciprocal dilutions of monoclonal antibody binding to heterologous vs homologous myeloma protein subclasses at 5%, 20%, and/or 50% of the maximum optical density ( $OD_{max}$ ) was used as a measure of cross-reactivity. Specificity of the HP-series of monoclonal antibodies is summarized in Table 4. Results obtained in these analyses agree well with similar studies performed at the CDC (89) and in the IUIS/WHO collaborative study in laboratories using ELISA and immunofluorometric assays (13, 95). Dilution curves generated in a representative cross-reactivity study of HP6025 (anti-human IgG<sub>4</sub> Fc) are presented in Figure 3.



**Figure 3.** Determination of specificity by dilution analysis. Thirteen dilutions of HP6025 were analyzed in an ELISA by using microtiter wells coated with human IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub> or IgG<sub>4</sub> myelomas. Cross-reactivity was defined as the ratio of ascites dilution that produced the same optical density after binding of monoclonal antibody to homologous (IgG<sub>4</sub> myeloma) vs heterologous IgG subclass (IgG 1, 2, or 3).

# Quantitation of Antibody

The quantity of immunoreactive monoclonal antibody in ascites was analyzed by ELISA (96). Microtiter plate wells were coated with one of four human IgG subclass myelomas or with bovine serum albumin (BSA, negative control). Dilutions of antibody in ascites and purified form were incubated in replicate wells coated with human IgG-subclass myelomas. Bound murine antibody was then detected by means of enzyme-conjugated polyclonal anti-serum to mouse IgG (preabsorbed against human IgG) and developed with substrate. Net optical density was plotted as a function of the reciprocal dilution of ascites or nanograms per ml of purified antibody standard. Parallel ascites dilution curves obtained in a potency study are presented in Figure 4. The quantity of antibody was determined either (A) in weight per volume units by interpolation from a dose-response curve produced by using a chromatographically purified preparation of the same monoclonal antibody with known concentration (mg/ml) of antibody (standard), or (B) in arbitrary units as a ratio of the dilution of the test sample vs a reference sample at 50% maximum optical density. Approach B was used only in the screening of the culture medium and initial evaluation of ascites for the monoclonal antibody of interest.

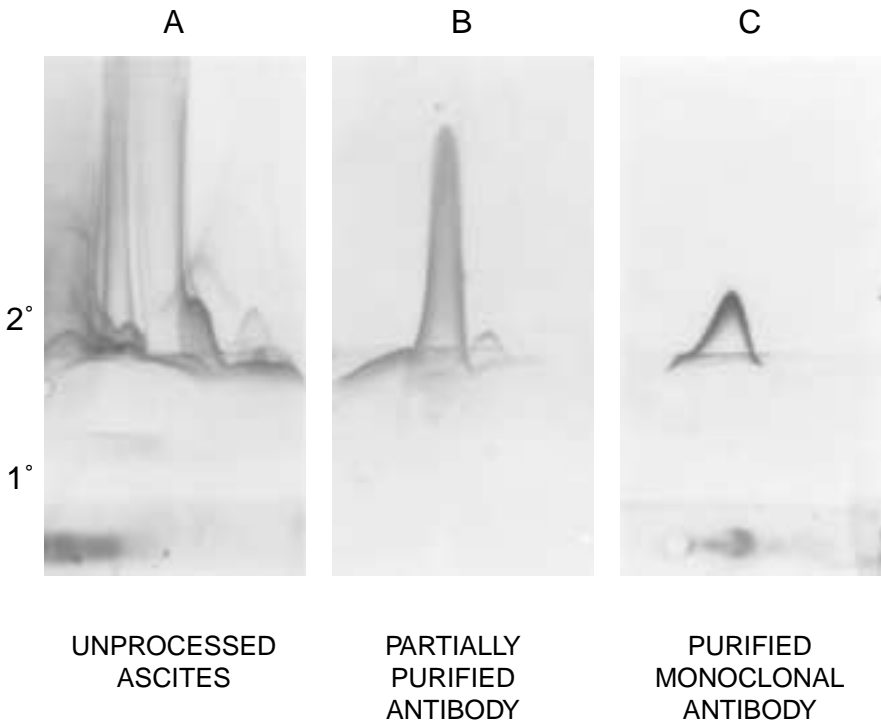


**Figure 4.** Determination of potency by dilution analysis. Binding curves are shown for 13 dilutions of six monoclonal antibodies (HP6017-aGFc, HP6046-aGFd, HP6001-aG1, HP6002-aG2, HP6050-aG3, and HP6025-aG4) analyzed on human IgG PAN (1, 2, 3, 4)-coated microtiter wells. The potency of antibody in each ascites sample was defined as the reciprocal dilution at 50% maximum response (%OD<sub>max</sub>). Results from these studies are summarized in Table 3. Reproduced with permission from (92).

## Purity of Isolated Antibody

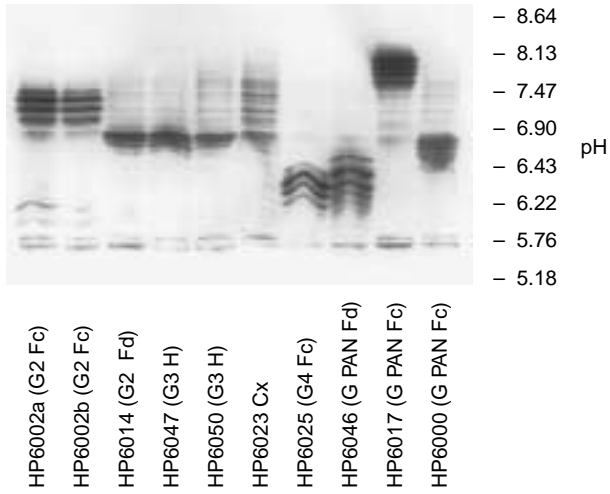
Purity of each lot of monoclonal antibody was assessed by IEF and/or crossed-immunoelectrophoresis (XIE). XIEs of unprocessed and partially purified ascites and of purified monoclonal antibody are reproduced in Figure 5.

IEF analysis also permitted routine quality control of each lot of antibody in terms of its relative purity. Ascites generally contained variable amounts of polyclonal host mouse IgG as shown by direct immunoblot analysis of the ascites after IEF (Figure 6).

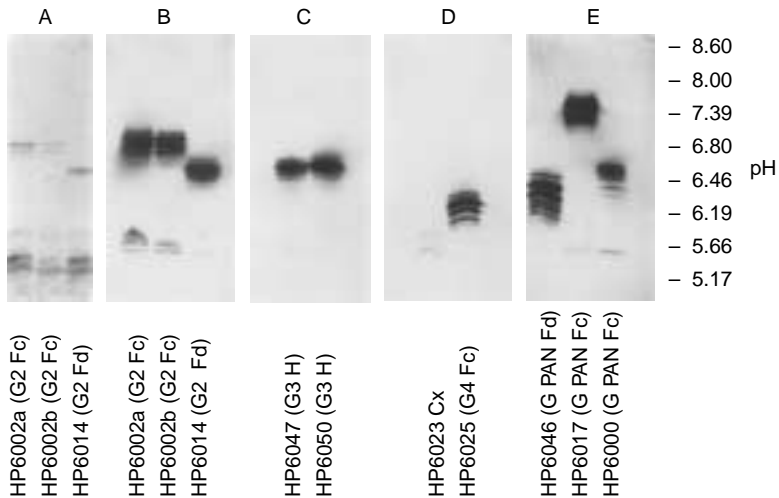


**Figure 5.** Crossed-immunoelectrophoresis analysis of mouse antibody to human IgG Fd (HP6045). Unprocessed ascites (panel A), partially purified antibody (panel B) and chromatographically purified monoclonal antibody (panel C) were separated by electrophoresis in a first dimension (1°) in agarose, cut out, inserted into a second gel and subjected to electrophoresis in a second dimension (2°) into a gel containing goat antiserum to mouse immunoglobulin. The height of each band reflects the relative quantity of IgG, and the number of bands relate to the purity. Panel A shows multiple peaks that indicate the presence of major quantities of albumin, transferrin, and other mouse proteins in addition to mouse IgG. The partially purified antibody in panel B contains a small amount of transferrin contaminant. Panel C shows the purity of the chromatographically purified mouse IgG and monoclonal antibody (HP6045), which is free from other host protein contaminants.





**Figure 6.** Direct immunoblots for detection of mouse IgG of ascites. Ten murine ascites samples were focused isoelectrically in a polyacrylamide gel that was subsequently overlaid with untreated nitrocellulose paper. Bound murine IgG was detected with peroxidase-conjugated antiserum to mouse IgG and developed with substrate. Polyclonal murine IgG in the ascites displayed a heterogeneous pI range from pH 5.5-8.0. Major (dense) bands are the monoclonal antibody (typically 2-5 mg/ml) shown in the IEF affinity immunoblots in Figures 6 and 7. Reproduced with permission from (92).

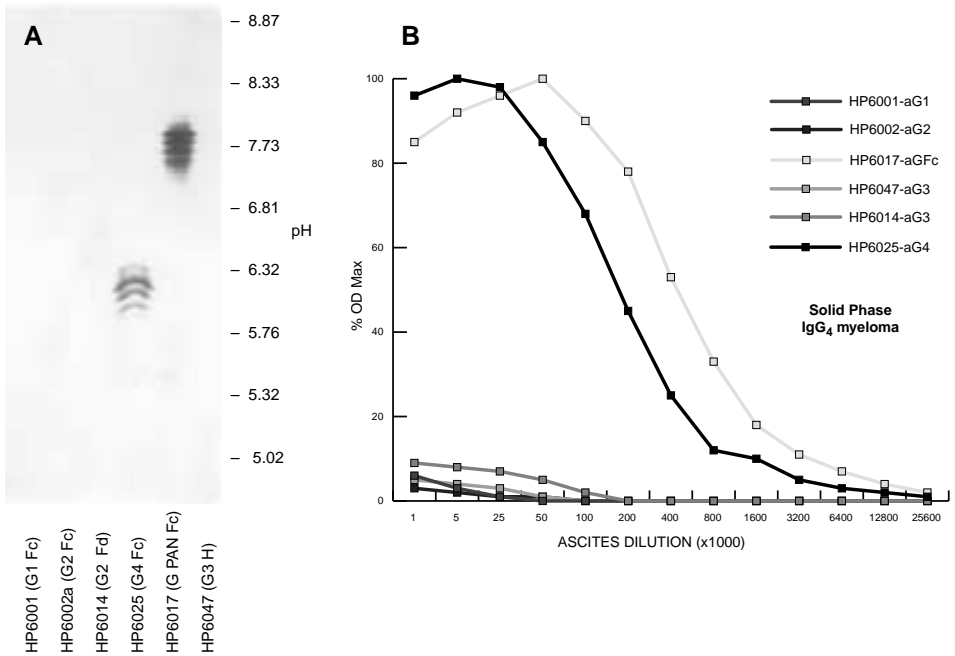


**Figure 7.** IEF-affinity immunoblot of human IgG specific monoclonal antibodies for definition of each antibody's pI pattern (band, number). The monoclonal antibody to human-IgG-containing ascites were subjected to IEF by using ampholytes (pH range 4-9). The IEF gel was blotted with nitrocellulose coated with human IgG<sub>2</sub> myeloma pool (panel B), human IgG<sub>3</sub> myeloma pool (panel C), human IgG<sub>4</sub> myeloma pool (panel D), and human IgG<sub>1-4</sub> polyclonal human IgG (panel E). Bound mouse IgG antibody was then detected with peroxidase-conjugated anti-mouse IgG and developed with substrate. Panel A is the Coomassie-blue-stained polyacrylamide gel after affinity immunoblotting that can be compared directly with the IEF-affinity immunoblot in panel B. Each monoclonal antibody produced a unique immunoblot fingerprint that can be used in quality control of future ascites lots. Reproduced with permission from (92).

Polyclonal murine IgG in the ascites produced bands with pIs ranging from 5.5 to 8.0. Monoclonal antibodies specific for the human IgG subclasses were identified on IEF-affinity immunoblot by using IgG-myeloma-coated nitrocellulose paper (Figure 7).

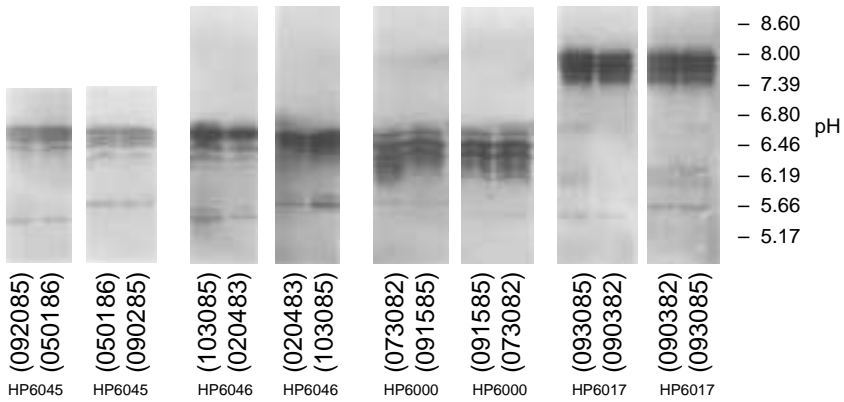
Each antibody was characterized by its unique IEF “fingerprint,” which spanned 0.4 – 0.8 pH units and was composed of one to five major (dense bands) and several minor (faint) bands. Use of human IgG-myeloma-coated nitrocellulose allowed initial assessment of the specificity of the monoclonal antibody in ascites (Figures 8A and 8B).

The IEF banding pattern of each antibody is described in Table 3 in terms of the pI range and number of major and minor bands. Semiquantitative speci-



**Figure 8A.** Comparative specificity assessment of the HP-series of human IgG specific monoclonal antibodies by using an IEF-affinity immunoblot (panel A) and ELISA (panel B). Samples of ascites containing antibody were subjected to IEF by using ampholytes (pH range 4-9). Nitrocellulose paper was coated with human IgG<sub>4</sub> (panel A) myeloma, blocked with PBS-BSA and laid onto the IEF gel. Bound mouse antibody was then detected by using peroxidase-conjugated antiserum to mouse IgG Fc and visualized with substrate.

**Figure 8B.** Panel B displays the ELISA cross-reactivity dilution curves obtained with the same ascites analyzed by the IEF-affinity immunoblot in panel A. Microtiter plates were coated with IgG<sub>4</sub> and processed as discussed in the methods. Complementary data on each antibody was produced by the two methods with regard to the antibody's net charge or pI, potency, and specificity. Reproduced with permission from (92).



**Figure 9.** IEF-affinity immunoblot analysis of the HP-series of human-IgG-specific mouse monoclonal antibody for determination of immunoreactivity and pI fingerprints (pI range, band number). Two lots of ascites from four hybridoma cell lines (prepared 1-3 years apart) were subjected to IEF, and human IgG specific antibodies were bound through passive diffusion onto human IgG coated on nitrocellulose. Bound mouse IgG was and substrate. Lot-to-lot variation among ascites was studied by comparing IEF-affinity immunoblot patterns of two lots of ascites produced from four hybridomas in replicate runs. IEF immunoblot patterns demonstrated consistency between pI fingerprints obtained in separate gels with two lots of ascites. Reproduced with permission from (93).

ficity results obtained in IEF-affinity immunoblot agreed well with the quantitative cross-reactivity results generated by ELISA. The IEF-affinity immunoblot is used for quality control of new lots of ascites produced by the same clone over extended periods of time (Figure 9).

## IV. Applications

Murine monoclonal antibodies to human IgG and its four subclasses have been applied in two major areas of investigation: (1) quantitation of the level of each IgG subclass in serum and (2) determination of the subclass(es) of human IgG antibodies. Other applications of these antibodies involve the subtyping of human IgG myeloma paraprotein with electrophoresis immunofixation and isolation of IgG subclasses from serum by means of affinity chromatography.

### Immunoassays of Human IgG Subclasses

Quantitation of the amount of each IgG subclass in a given serum sample has allowed identification of selective IgG subclass deficiencies and myeloma states. It has also been useful in monitoring intravenous immunoglobulin replacement, plasmapheresis, and immunosuppression therapy. Normal range of the four human IgG subclasses have been documented in several studies primarily in Caucasian and Black adults (54, 97-104) and children (62, 64-66, 99, 105-110). The methods used to measure the level of each IgG subclass in serum have involved immunodiffusion, immunoelectrophoresis, agglutination and, more recently, various forms of quantitative immunoassays. Moreover, assays for the quantitation of human IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, and IgG<sub>4</sub> are achieving a level of comparability throughout the world because of the availability of several characterized reference sera from the WHO (WHO Reference 67/97) (111), the CDC (U.S. Reference IS1644) (112) and the National Institute for Biological Standards and Control in London (British Reference: WHO 67/68) (113). The concentrations of human IgG subclasses in these preparations have been defined independently by several investigators using immunodiffusion or immunoassay methods (114, 115). The wide availability of monoclonal antibodies of defined specificity for the human IgG subclasses, such as the HP-series (Table 3), has made quantitative immunoassays for IgG subclasses more commonplace in clinical immunology laboratories.

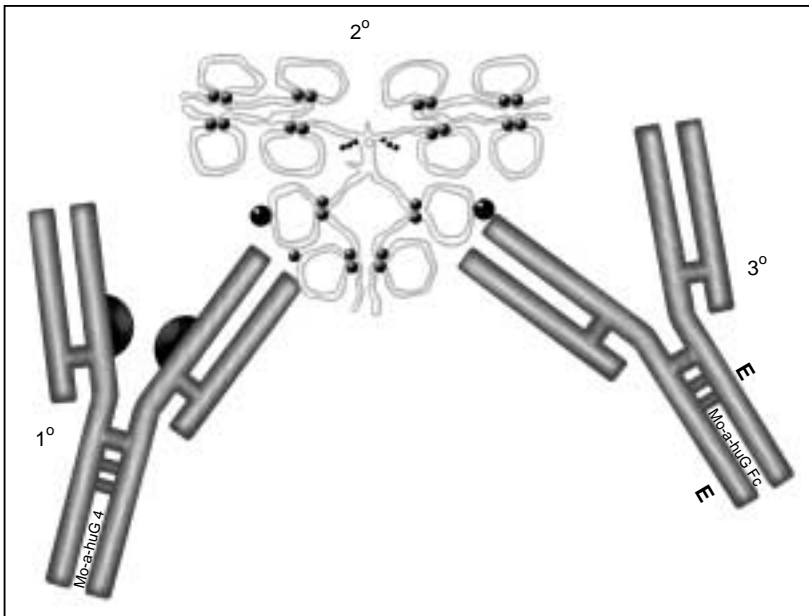
The most widely used immunoassay configuration for measurement of each IgG subclass is shown schematically in Figure 10. Human IgG subclass-specific monoclonal antibody is fixed onto a solid phase either by absorption or by covalent coupling. Absorption of monoclonal antibody onto microtiter plates can be accomplished best by using chromatographically purified antibody. Diluted ascites also has been used (94, 114); however, ascites proteins other than IgG may block limited binding sites on the plate.

A modification to this approach may be the use of an initial layer of purified polyclonal anti-mouse IgG to bind mouse IgG from an ascites sample onto a plastic surface with its human IgG binding regions extending into the liquid phase. This approach may increase the amount of immunoreactive mouse antibody to human IgG subclass bound to microtiter plates; however, cross-reactivity between the goat anti-mouse IgG and human IgG must be eliminated to minimize nonspecific binding. Optimal coating conditions vary as a function of the type of solid phase; pH and ionic strength of the coating buffer; and the concentration, subclass and pI of the murine antibody. Coating

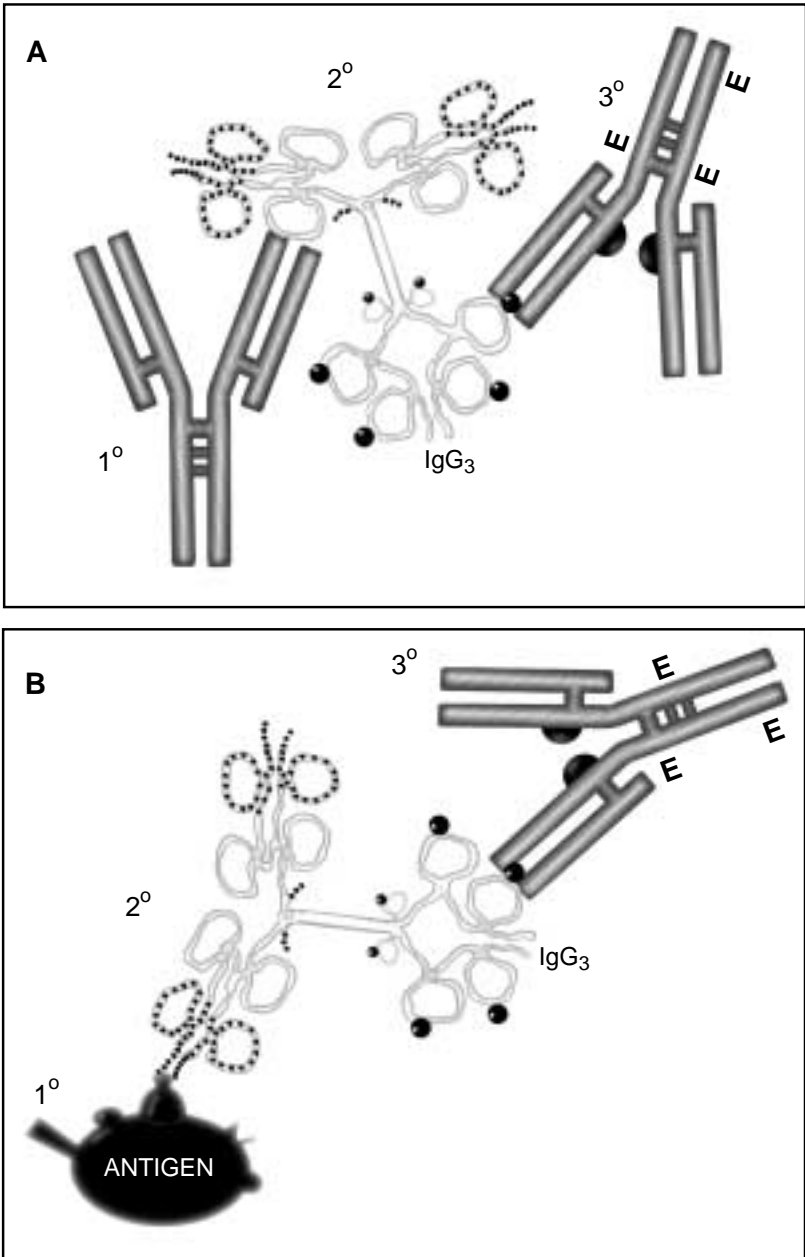
of the HP-series of antibodies to Immulon II microtiter plates (Dynatech) occurs maximally at 3 – 5 µg/ml of chromatographically purified antibody in PBS (pH 7.4) with 2 – 4 hours at +37°C or overnight incubation at +23°C or +4°C.

Once the capture antibody has been bound to the solid phase, blocking is commonly performed by using irrelevant protein. Dilutions of human reference or test serum then are incubated with the solid phase monoclonal anti-human IgG (e.g., 2 – 4 hours, 37°C). A dose-response curve is generated by using multiple dilutions of human serum standards (WHO 67/68; WHO 67/97; and CDC IS1644, Lot 12-0575c). Removal of unbound human IgG is accomplished with buffer washes, and bound human IgG is detected with enzyme-conjugated polyclonal or monoclonal antibodies to human IgG (see Appendix I) that have been adsorbed against mouse IgG. Measurements of absorbance generated by substrate in the test sera can be interpolated from the dose-response curve to define the amount of the IgG subclass in the serum.

Several reports have discussed the use of the HP-series monoclonal antibodies for the measurement of the human IgG subclasses by using minor variations on the general assay format discussed previously and depicted in Figure 10 (92, 114, 116). Serum dilution curves obtained with these antibodies in one recommended enzyme immunoassay format are presented in Appendix I. Good



**Figure 10.** Schematic diagram of the immunoassay for total human IgG<sub>4</sub>. Monoclonal anti-human IgG<sub>4</sub> Fc (HP6023) is coated on microtiter plates. Once blocked with BSA, dilutions of test or reference human sera are pipetted into their respective wells, and IgG<sub>4</sub> is bound to the plate. Unbound IgG is then removed, and bound IgG<sub>4</sub> is detected with enzyme-conjugated antiserum to human IgG and substrate. A dose-response curve is generated by using primary or secondary standards in each ELISA to allow interpolation of test sera response data in weight per volume units of human IgG<sub>4</sub>.



**Figure 11.** Schematic diagram of the immunoassay for antigen-specific IgG<sub>3</sub> antibody. Antibodies of all classes bind to antigen that has been attached to microtiter plates (panel B). Bound IgG<sub>3</sub> antibody is then detected with monoclonal antibody to human IgG<sub>3</sub> that has been either enzyme-labeled or subsequently detected with labeled polyclonal antiserum to mouse IgG. A heterologous dose-response curve can be generated to standardize the assay (panel A). Known amounts of human IgG<sub>3</sub> are bound by insolubilized antibody to human IgG Fd or a different monoclonal antibody to human IgG<sub>3</sub>. Bound IgG<sub>3</sub> then is detected with the conjugated antibody human IgG<sub>3</sub> used in panel B. This approach of heterologous interpolation can be used until an IgG<sub>3</sub> antibody reference preparation is made available (96).

parallelism was observed between the CDC IS1644 reference serum and four test human sera when HP6046 (anti-human IgG Fd PAN), as the capture antibody, and horseradish peroxidase conjugated HP6017 (anti-human IgG Fc PAN) were used in the total IgG assay. Parallel dilution curves also were obtained in the IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub> and IgG<sub>4</sub> ELISAs when the CDC and WHO 67/97 reference sera were compared. IgG subclass levels obtained in these assays with human sera analyzed at multiple dilutions are presented in Table 5. Use of an IgG subclass standard; control sera containing high, medium, and low IgG subclass concentrations; and test sera in at least three dilutions in replicate produce high quality total IgG subclass measurements.

## Immunoassay of IgG Subclass Antibody

An extensive literature has been developed on the distribution of subclasses in IgG antibodies produced during immune responses to bacterial, viral, and parasitic antigens; autoantigens; tumor antigens; and many parenterally administered substances such as hormones, drugs, and allergens (see literature survey). Table 6 summarizes published subclass distributions of IgG antibodies detected against bacterial and viral antigens. In adults, two distinct IgG subclass patterns are induced by either the bacterial protein or polysaccharide antigens (87). Bacterial proteins mostly induce IgG<sub>1</sub> antibodies, with minor levels of IgG<sub>3</sub> and IgG<sub>4</sub> antibodies. Polysaccharide antigens mostly induce IgG<sub>2</sub> antibodies. IgG<sub>2</sub> antibodies also are produced if human B cells are stimulated *in vitro* by pneumococcal capsular polysaccharides (117, 118). Moreover, children produce antibodies to bacterial carbohydrate antigen of the IgG<sub>1</sub> class that generally are not found in adults. In contrast, IgG antibodies to viruses are highly restricted to IgG<sub>1</sub> and IgG<sub>3</sub>, with IgG<sub>3</sub> antibodies appearing first in the course of an infection (88). Early in parasitic and allergic diseases, IgG immune responses to protein antigens appears to be primarily IgG<sub>1</sub> antibodies, with low levels of IgG<sub>3</sub> and IgG<sub>4</sub> antibodies. After hyperimmunization, however, IgG<sub>4</sub> antibodies increase, and the immune response may become restricted to subclass 4 (71, 119).

Restriction of an immune response to one or several IgG subclasses can be studied by using minor modifications of the total IgG assay outlined. Solid phase antigen is used to bind specific antibody, and the subclass of bound IgG is detected with subclass-specific monoclonal antibodies (Figure 11).

The HP-series of anti-human IgG monoclonal antibodies have been used unconjugated with a second antibody (anti-mouse IgG) or directly conjugated to horseradish peroxidase or alkaline phosphatase and used directly as a detection antibody for the subclasses of human IgG (see Appendix I, Monoclonal Antibody Conjugates). The most challenging aspect of measurement of IgG subclass antibodies resides in quantifying what amount of IgG antibody is of IgG subclass 1, 2, 3 or 4 for purposes of comparing their levels and studying changes in subclass distribution over the course of a disease or immunization. Quantitation of the amount of each subclass antibody as a part of the total IgG immune response to a particular antigen is complicated by the lack of defined standards (71, 120, 121). A discussion of methods for standardization of the antigen-specific IgG subclass assays is beyond the scope of this monograph (122-124).

# V. Literature Survey

This survey of the literature is divided into two sections. The first section focuses on published reports relating to general properties of the human IgG subclasses. The second examines the literature by disease category. It directs the reader to the distribution of the IgG subclasses in defined diseases. The reports discussed in this survey all relate to the structure, biological properties, or distribution of human IgG and its subclasses in humans. No attempt has been made to segregate those studies that use monoclonal antibodies from reports that use polyclonal antibodies as assay reagents. Special reviews are denoted by parentheses.

## General Aspects of the Human IgG Subclasses

*Allotypes* and genes (23, 24, 28, 30-42).

*Affinity* and Immune System Maturation (125-128).

*Antigenic determinants* on the IgG subclasses (4, 6, 8, 9, 129-134).

*Assay methods:*

Enzyme immunoassay (92-94, 114, 135-142).

Nephelometry, turbidimetry and particle counting (143-146).

Radioimmunoassay (147-149).

Standardization and methodology (96, 115, 150-154).

*B-cell* lymphocyte function and stimulation (155-168).

Antibodies that *block* immediate hypersensitivity (see allergy section under Disease Category).

*Carbohydrates* on Human IgG Subclasses (169-174).

*Catabolism* of the human IgG subclasses (56, 175, 176).

*Cell surface* IgG subclasses (177, 178).

*Complement activation* (70, 128, 179-189).

IgG (subclasses) produced in *cell culture* (159, 162, 164, 168, 190-192).

IgG subclass *concentration* in infancy and childhood (58, 62, 64-67, 99, 105-110, 193).

IgG subclass *concentration* in adults (54, 55, 97-104).

IgG subclass *concentration* in amniotic fluid and serum cord (107, 194).

IgG subclass *concentration* in cerebral spinal fluid (195-199).

IgG subclass *concentration* in colostrum (107, 200, 201).

IgG subclass *concentration* in the intraocular compartment (202).

*Conformation* and structure of human IgG subclasses (3, 18, 21, 132, 203-213).

*Cross-Species* homology (214).

*Cryoglobulins* (215-218).

Interaction with *cystic fibrosis factor* (83, 231)

*Effector* functions (69, 72, 172, 180, 181, 187, 219-227).

*Humanized* and chimeric (human-mouse) antibodies (123, 124, 171, 172, 185-188, 227-230).

*Electrophoretic* analysis of IgG subclasses (45, 46, 232, 233).

*Factor VIII and IX* antibodies (234-237).

*Fragmentation* of the IgG subclasses (22, 47, 48, 50-52, 133, 206, 209, 224, 238-242).



*Hybrid IgG<sub>2</sub>-IgG<sub>4</sub> immunoglobulin* (243).  
*IgA subclass secretion and interactions with lectins* (243-247).  
 Properties of *IgG<sub>1</sub>* (18, 71, 134, 180, 197, 248, 249).  
 Properties of *IgG<sub>2</sub>* (6, 29, 133, 180, 211, 250-256).  
 Properties of *IgG<sub>3</sub>* (21, 134, 219, 239, 257-263).  
 Properties of *IgG<sub>4</sub>* (71, 101, 135, 252, 264-271).  
*Immunoglobulin genes* (deletion, other defects, HLA associations, mRNA, hybridization techniques) (272-279).  
*Isolation methods* (9, 280-286).  
*J chain* (287, 288).  
*Light chain* (139, 144, 289-291).  
 Interaction with *lymphocytes* (72, 75, 177, 178, 292-295).  
*Monoclonal antibodies to the human IgG subclasses and allotypes* (8, 12, 13, 89, 92, 139, 207, 291, 296-298).  
 Interaction with *monocytes*, macrophages, neutrophils and eosinophils (73-75, 299-308).  
 Human *mucosa* and parotid gland (309,310).  
 Human IgG subclass *myeloma* proteins (18, 43, 44, 79, 132, 144, 253, 311-317).  
*Polyclonal antibodies to the human IgG subclasses* (318-323).  
*Primary-secondary immune responses* (324, 325).  
 Interaction with *S. aureus Protein A* (82, 283, 285, 286, 326).  
 Interaction with *Protein G* (282, 326).  
*Quality control* of antibodies specific for human IgG subclasses (8, 89, 93).  
*Fc receptors interactions* (72, 219, 221, 227, 301, 327-334).  
*Reference immunoglobulin preparations* (111-113, 335-337).  
*Reviews on the IgG subclasses* (15, 53, 100, 338-342).

## Literature Survey by Disease Category

### Allergy and Pulmonary Diseases:

*Anti-IgE autoantibodies* (330, 343-345).  
 IgG subclasses in *atopy* and asthma (40, 101, 127, 271, 346-353).  
 Histamine release from *basophils* with anti-IgG<sub>4</sub> (117, 330, 354, 355).  
 Subclasses of IgG antibodies specific for *dextran* (356, 357).  
*Drug allergy* ( $\beta$ -lactam ring) (358, 359).  
 IgG subclasses in *Farmer's lung* and aspergillosis (360-362).  
 IgG subclasses in *food allergy* (120, 363-370).  
 Hay fever patients on *grass pollen* immunotherapy (376-378).  
*Hyper-IgE syndrome* (371, 372).  
 IgG antibodies in immunotherapy (71, 264, 373-375).  
*House dust mite allergy* (94, 379).  
*Hymenoptera venom allergy* (265, 379-382).  
*Reversible airway obstruction* (385-386).  
*Serum sickness* (383, 384).

### Autoimmunity:

*Anti-basement membrane zone autoantibody* in pemphigoid (387-392).  
*Chronic fatigue syndrome* (440, 441).  
*Collagen II autoantibodies* (393, 394).

Anti-*islet cell* cytoplasmic antibodies in diabetes (395, 396).  
IgG subclasses of anti-*DNA* (399-404).  
Anti-*insulin* antibodies (405-408).  
*Phospholipid* autoantibodies (409, 410).  
IgG antibodies in *rheumatoid arthritis* (411-415).  
*Rheumatoid factors* reactivity with human IgG subclasses (85, 190, 229, 416-420).  
*IgG rheumatoid factors* (84, 182, 421-423).  
IgG subclasses of anti-*ribonucleoproteins* (397-399).  
Antibodies in *scleroderma* (424, 425).  
Anti-*sperm* (426, 427).  
Antibodies in *systemic lupus erythematosus* (398, 401, 402, 410, 428, 429).  
*Thyroid* and TSH receptor-related autoantibody (179, 430-439).

### **Connective Tissue Disease:**

IgG subclass antibodies in *acne vulgaris* and *bullous pemphigoid* (442-444).  
IgG subclasses in *ankylosing spondylitis* (445).  
Subclasses of IgG antibodies in *sarcoidosis* (446).  
IgGs in *vasculitis* (126).

### **Gastroenterology:**

*Coeliac* disease (447, 448).  
Ulcerative *colitis* (449, 450).  
*Helicobacter pylori* Infection in gastritis and ulcer (451, 452).  
*Intestinal* (distal ileum) and lymph follicle B cells (288, 453-455).  
*Liver cirrhosis* (456, 457).

### **Hematology:**

Sickle cell *anemia* (458-461).  
Anti-*D (Rho)* and anti-*P* antibodies (268, 466-471).  
*Hemophilia* (234, 462, 463).  
*Hemolytic disease* in newborns (464, 465).  
*Placental transmission* of IgG subclasses (57, 59, 60, 63, 476-479).  
Anti-*platelet* antibodies (76, 480-484).  
IgG subclass antibodies to *red blood cells* (74, 472-475).

### **Primary Immunodeficiency and SCID model mouse (259, 485-490):**

*Acquired* immune deficiency syndrome (AIDS) (251, 257, 491-498).  
*Deficiency* of selected subclasses (233, 499-507).  
*Hypogammaglobulinemia* (508-513).  
*Immunodeficiency* and recurrent infection (514, 515).  
IgG subclass levels in *intravenous immunoglobulin* preparations (108, 516-520).

### **Infection and Immunization (86-88, 521-523):**

IgG subclass antibodies in *brucellosis* (524, 525).  
*Campylobacter jejuni*-specific antibodies (526).  
*Cytomegalovirus* specific antibodies (527-529).  
IgG subclass antibodies in *filariasis* (119, 267, 530-533).

IgG anti-*Hemophilus influenzae* polysaccharides (117, 180, 534-536).  
IgG antibodies specific for *hepatitis B* surface antigen and hepatitis C core protein (537-543).  
IgG subclasses in *leprosy* (544, 545).  
Antibodies to *P. falciparum malaria* (546-548).  
IgG subclasses in *measles* (549-551).  
IgG subclasses of *mumps* antibodies (552).  
*Pneumococcal* polysaccharide immune responses (553-559).  
*Pseudomonas* lipopolysaccharide antibody and binding proteins (27, 560-565).  
*Recurrent infections* associated with immunodeficiency (254, 492, 566-571).  
Subclasses of IgG *rubella* antibodies (572-574).  
IgG subclasses of antibodies to *Salmonella* (575-578).  
IgG subclasses in *schistosomiasis* (86, 308, 579, 580).  
*Staphylococcus aureus* infections (87, 180, 371, 581).  
Antibodies to *streptococci* (87, 332, 582-586).  
*Syphilis* (587, 588).  
IgG subclasses of *treponemal* antibodies (589, 590).  
Immune responses to *tetanus toxoid* (154, 266, 591-593).  
IgG subclasses to *toxic shock syndrome* toxin (594, 595).  
Antibodies to *Trichuristrichiura* (596, 597).  
IgG subclasses of *viral* antibody (88, 137, 293, 529, 550, 598-605).

### **Nephrology** (606):

*Diabetic kidney* (269).  
Anti-basement membrane *nephritis* (607, 608).  
*Nephrotic syndrome* (609-614).

### **Neurology**:

Human IgG subclasses in the *central nervous system* (195, 615).  
Antibodies in *multiple sclerosis* (197, 375, 616, 617).  
IgG subclass antibodies in *myasthenia gravis* (618-620).  
*Optic neuritis* (621).

### **Oncology**:

IgG subclasses in *ataxia telangiectasia* (622-624).  
IgG subclasses in *breast cancer* (625, 626).  
Chronic lymphocytic *leukemia* (627, 628).  
*Lymphomas* in children (629-632).  
Malignant *melanoma* (633).  
Multiple *myeloma* (261, 312, 634-636).

### **Periodontal Disease**:

IgG subclasses in human *periodontal disease* (637-641).

### **Radiology**:

IgG subclass levels in patients receiving *radiation therapy* (642, 643).

**Table 1. Physical-Chemical Properties of the Four Human IgG Subclasses**

Properties	IgG <sub>1</sub>	IgG <sub>2</sub>	IgG <sub>3</sub>	IgG <sub>4</sub>
Heavy chain type	γ <sub>1</sub>	γ <sub>2</sub>	γ <sub>3</sub>	γ <sub>4</sub>
Molecular weight (x 1000)	146	146	170	146
Hinge amino acid number	15	12	62	12
Interchain disulfide bond number	2	4	11	2
Light chains: κ : λ ratio	2.4	1.1	1.4	8.0
pI range: mean ± 1 SD (46)	8.6 ± 0.4 n = 18	7.4 ± 0.6 n = 11	8.3 ± 0.7 n = 8	7.2 ± 0.8 n = 3

**Susceptibility to digestion (53)**

Papain (in the absence of cysteine)	+	+/-	+	+
Plasmin	+++	+	+++	+
Trypsin	+++	+	+++	+++
Pepsin	+	+	+++	+++
Subtypes	Gm <sub>1</sub>	Gm <sub>2</sub>	Gm <sub>3</sub>	Gm <sub>4</sub>
Number of Allotypes	4	1	13	0

**Table 2. Biological Properties of the Four Human IgG Subclasses**

Properties	IgG <sub>1</sub>	IgG <sub>2</sub>	IgG <sub>3</sub>	IgG <sub>4</sub>
Human myeloma protein frequency (%)	60-70	14-20	4-8	2-6
Proportion of total IgG in normal adult serum (%) (54)	60.3-71.5	19.4-31.0	5.0-8.4	0.7-4.2
Average serum concentration (mg/ml)	8	4	0.8	0.4
Range in normal serum (mg/ml) (2)	5-12	2-6	0.5-1	0.2-1
Subclass distribution on circulating B cells by immunofluorescence (%) (270)	40	48	8	1
Subclass distribution on IgG plasma cells in bone marrow, spleen, tonsil (%) (270)	64	26	8	1
Half-life (days)	21-23	20-23	7-8	21-23
Transport across the placenta	++	+	++	++
Complement fixation (classical pathway)	++	+	++	0
Antibody response to:				
Proteins	++	+/-	++	+/-
Polysaccharides	+	++	-	-
Allergens	+	-	-	++
Cytophilic properties*				
Human monocytes (FcR-I, II & I <sub>o</sub> )	++	+	++	+/-
Human neutrophils (FcR-II)	++	+/-	++	+
Human platelets (FcR-II)	++	+	++	+
Human lymphocytes (FcR-II or I <sub>o</sub> )	++	+/-	++	+
Nonimmunological Reaction with				
<i>S. aureus</i> protein A	+	+	0	+
Cystic fibrosis factor	+	+	0	0
Rheumatoid factors	+	+	0	0
Functional valency (70, 71)	2	2	2	2
Blocking activity in allergy	0	0	0	+
Binding to Protein A	++	++	Allotype dependent	++
Binding to Protein G	++	++	++	++

\*Three types of human IgG Fc receptors (FcR) can reside on human leukocytes (FcR-I, FcR-II and/or FcR-I<sub>o</sub> [low affinity]) (72).

**Table 3. Anti-Human IgG Monoclonal Antibodies**

Clone Designation	Mouse Isotype	Specificity for Human IgG	Potency for IgG (x10 <sup>5</sup> )	pI Range and (Mean)**	Major:Minor IEF Bands
HP6000	IgG <sub>2b</sub>	PAN IgG Fc	1.3	6.0–6.5 (6.3)	5M:4m
HP6017	IgG <sub>2a,κ</sub>	PAN IgG Fc	4.5	7.4–7.8 (7.6)	4M:4m
HP6045	IgG <sub>2a</sub>	PAN IgG Fd	0.95	6.3–6.7 (6.5)	4M:2m
HP6046	IgG <sub>1</sub>	PAN IgG Fd	3.0	6.1–6.6 (6.3)	3M:5m
HP6062	IgG <sub>3</sub>	κ (Kappa)	1.5	6.3–6.8 (6.5)	2M:3m
HP6054	IgG <sub>2a,κ</sub>	λ (Lambda)	1.0	6.8–7.2 (7.0)	2M:2m
HP6001	IgG <sub>2b</sub>	IgG <sub>1</sub> Fc	1.3	7.6–8.2 (7.9)	3M:1m
HP6069	IgG <sub>1,κ</sub>	IgG <sub>1</sub> Fc		6.3–6.8 (6.4)	
HP6070	IgG <sub>1,κ</sub>	IgG <sub>1</sub> Fc		6.1–6.4 (6.2)	
HP6091	IgG <sub>2a,κ</sub>	IgG <sub>1</sub> Fc			
HP6002	IgG <sub>1</sub>	IgG <sub>2</sub> Fc	1.5	6.8–7.4 (7.1)	4M:2m
HP6014	IgG <sub>1,κ</sub>	IgG <sub>2</sub> Fd	5.1	6.5–6.7 (6.6)	3M:1m
HP6047	IgG <sub>1</sub>	IgG <sub>3</sub> hinge	9.1	6.5–6.7 (6.6)	3M:2m
HP6050	IgG <sub>1,κ</sub>	IgG <sub>3</sub> hinge	1.3	6.5–6.9 (6.7)	3M:2m
HP6023	IgG <sub>3,κ</sub>	IgG <sub>4</sub> Fc	1.0	7.5–7.9 (7.7)	3M:3m
HP6025	IgG <sub>1,κ</sub>	IgG <sub>4</sub> Fc	1.7	6.2–6.5 (6.3)	3M:3m

\*The potency of relative amount of antibody in the ascites is presented as the reciprocal of the dilution that produces 50% maximum optical density.

\*\*pI range and mean for each monoclonal antibody are presented as determined by IEF-affinity immunoblotting (92). The IEF pattern of each monoclonal antibody generally is composed of major (dense) and minor (faint) bands.

**Table 4. Anti-Human IgG Monoclonal Antibody\* Specificity Analysis**

Clone Designation	Reported Specificity	Human Myelomas				IgG PAN	Fragments	
		IgG <sub>1</sub>	IgG <sub>2</sub>	IgG <sub>3</sub>	IgG <sub>4</sub>		IgG Fc	IgG Fab
HP6000	IgG Fc	100	100	99	100	100	99	<0.15
HP6403	IgG Fc	100	100	100	100	100	100	<0.10
HP6017	IgG Fc	100	100	100	100	100	100	<0.12
HP6045	IgG Fd	100	100	100	99	100	0.04	100
HP6046	IgG Fd	100	99	100	100	100	0.06	100
HP6001	IgG <sub>1</sub> Fc	100	0.25	<0.01	<0.01	+	+	0
HP6069	IgG <sub>1</sub> Fc	100	0.1	0.01	0.01			
HP6070	IgG <sub>1</sub> Fc	100	0.2	0.1	0.02			
HP6091	IgG <sub>1</sub> Fc	100	0.1	0.2	0.05			
HP6002	IgG <sub>2</sub> Fc	<0.01	100	<0.01	<0.01	+	+	0
HP6014	IgG <sub>2</sub> Fab	0.08	100	0.8	0.06	+	0	+
HP6050	IgG <sub>3</sub> Fab	<0.01	<0.01	100	<0.01	+	0	+
HP6047	IgG <sub>3</sub> Fab	0.04	0.9	100	<0.01	+	0	+
HP6023	IgG <sub>4</sub> Fc	0.08	<0.01	0.18	100	+	+	0
HP6025	IgG <sub>4</sub> Fc	0.48	0.08	0.18	100	+	+	0

Human Myelomas			
Clone Designation	Reported Specificity	κ	λ
HP6062	IgG κ	100	0.03
HP6054	IgG λ	0.2	100

\*Results are presented as a percent cross-reactivity as determined by dilutional analysis of monoclonal antibodies in ELISA (see methods). Results of monoclonal antibody binding to the IgG Fc and Fab fragments of human IgG subclass myeloma proteins were extracted from a report by Reimer, et al. (89).

**Table 5. Total IgG Levels in Human Serum**

<b>Specimen Code</b>	<b>Serum Dilution (x1000)</b>	<b>Interpolated Dose (mg/ml)</b>	<b>Dose (ng/ml) Dilution Corrected</b>	<b>(N)</b>	<b>Mean Dose</b>	<b>STD</b>	<b>ID %CV</b>
<b>Total IgG PAN (HP6046 capture antibody)</b>							
KW	16	983	15.73	4	14.75	1.62	11.0
	32	449	14.36				
	64	246	15.74				
	128	103	13.18				
MK	8	884	7.07	4	7.24	0.58	8.1
	16	420	6.72				
	32	232	7.42				
	64	121	7.75				
RH	16	470	7.52	3	7.36	0.21	2.86
	32	231	7.39				
	64	112	7.17				
<b>Total IgG subclass 1 (HP6001 capture antibody)</b>							
KW	4	2,155	8.62	4	7.17	1.36	19.0
	8	791	6.33				
	16	395	7.20				
	32	204	6.53				
MK	4	1,337	5.35	4	4.79	0.50	10.5
	8	556	4.53				
	16	287	4.59				
	32	146	4.67				
RH	2	3,128	6.26	5	5.61	0.92	16.3
	4	1,564	6.25				
	8	669	5.60				
	16	304	4.86				
	32	159	5.08				
<b>IgG subclass 2 [HP6002-HP6014 capture antibody mixture (1:1)]</b>							
KW	4	1,292	5.17	5	4.85	0.65	13.4
	8	599	4.79				
	16	280	4.48				
	32	136	4.35				
	64	85	5.44				
MK	4	520	2.08	4	2.20	0.28	12.8
	8	252	2.02				
	16	138	2.21				
	32	78	2.50				
RH	4	417	1.67	4	1.85	0.28	15.1
	8	209	1.67				
	16	124	1.98				
	32	65	2.08				



**Table 5. Total IgG Levels in Human Serum (cont'd.)**

Specimen Code	Serum Dilution (x1000)	Interpolated Dose (ng/ml)	Dose (mg/ml) Dilution Corrected	(N)	Mean Dose	STD	ID %CV
<b>IgG subclass 3 [HP6047-HP6050 capture antibody mixture (1:1)]</b>							
KW	4	175	0.70	3	0.55	0.16	28.9
	8	60	0.48				
	16	29	0.46				
MK	2	496	0.99	2	0.86	0.19	22.3
	4	179	0.72				
RH	2	399	0.80	2	0.70	0.14	20.2
	4	149	0.60				
<b>IgG subclass 4 [HP6025-HP6023 capture antibody mixture (1:1)]</b>							
KW	4	146	0.59	4	0.56	0.06	10.7
	8	68	0.54				
	16	38	0.61				
	32	16	0.51				
MK	8	14	0.11	2	0.14	0.04	26.2
	16	10	0.16				
RH	16	12	0.19	2	0.24	0.07	29.5
	32	9	0.29				

**Summary**

Patient	IgG <sub>1</sub> (mg/ml)	IgG <sub>2</sub> (mg/ml)	IgG <sub>3</sub> (mg/ml)	IgG <sub>4</sub> (mg/ml)	Sum 1-4 (mg/ml)	Total Measured	SUM MEASURED
KW	7.17	4.85	0.55	0.56	13.13	14.75	89.0%
MK	4.79	2.20	0.86	0.14	7.99	7.24	110.3%
RH	5.61	1.85	0.70	0.24	8.40	7.36	114.1%

N = number of dilutions analyzed

STD = inter-dilutional standard deviation of the mean antibody concentration

ID%CV = inter-dilutional percent coefficient of variation (STD/mean dose); a measure of immunoassay parallelism. An ID%CV < 20% is an indicator of acceptable assay parallelism (96).

**Table 6. IgG Subclasses in Specific Immune Responses**

Antigen Source	Test Population	IgG <sub>1</sub>	IgG <sub>2</sub>	IgG <sub>3</sub>	IgG <sub>4</sub>
IgG antibodies to bacterial proteins (87)					
<i>Salmonella</i> group B O antigen	c	++	-	-	-
<i>Streptococcus pneumoniae</i> type 3	c	++	+	-	-
<i>Hemophilus influenzae</i> membrane proteins	a	++	-	+	(-)
<i>Staphylococcus aureus</i> alpha toxin	a	++	+	-	+
<i>Streptococcus pyrogenes</i> pneumolysin	a	++	-	-	+/-
<i>Clostridium difficile</i> toxin A	a	++	-	-	-
IgG antibodies to bacterial polysaccharides (87)					
<i>Neisseria meningitidis</i> polysaccharide A	a	++	++	-	-
<i>Escherichia coli</i> lipopolysaccharide (LPS)	a	+/-	++	+/-	-
<i>Yersinia enterocolitica</i> LPS	a	+/-	++	-	-
<i>Staphylococcus aureus</i> teichoic acid	a	+	++	-	+
<i>Staphylococcus aureus</i> teichoic acid	c	++	+/-	-	-
<i>Streptococcus agalatae</i> polysaccharide B	a	+	++	-	-
<i>Leuconostoc mesenteroides</i> dextran B512	c	+	-	-	-
<i>Leuconostoc mesenteroides</i> dextran B512	a	+	++	-	-
IgG antiviral antibodies (88)					
Hepatitis Bs antigen		++	-	++	+/-
HTLV III (HIV)		++	+/-	+	+/-
Rubella virus		++	+/-	++	+
Measles and mumps virus		++	-	-	-
Influenza virus A and parainfluenza type I		++	+/-	++	++
Rotavirus subgroup 2		++	-	++	+/-
Cytomegalovirus		++	+/-	++	+/-
Epstein-Barr virus		++	-	-	-
<i>Varicella zoster</i> virus		++	-	++	+/-
<i>Herpes simplex</i> virus 1, 2		++	+/-	++	+/-
IgG antibodies induced by vaccination (341)					
<i>Clostridium tetani</i> (tetanus toxoid)		++	+/-	+	+
<i>Clostridium diphtheriae</i> (diphtheria toxin)		++	-	+/-	+
<i>Mycobacterium tuberculosis</i> (PPD)		++	-	-	-
<i>Vibrio cholerae</i> (endotoxin)		++	+/-	-	+/-
Rubella virus		++	+	++	+
Hepatitis B		++	+/-	+/-	+/-
Polio vaccine		ND	ND	++	ND

Data were summarized from references (87, 88, 341, 522). Note that the semiquantitative designations refer to study groups and any one patient can vary from the general trend. ++ = strong positive, + = positive, +/- = equivocal, - = negative; the test population is designated as adults (a) or children (c); ND = not done

**Table 7. Pathological Aspects of IgG Deficiency**

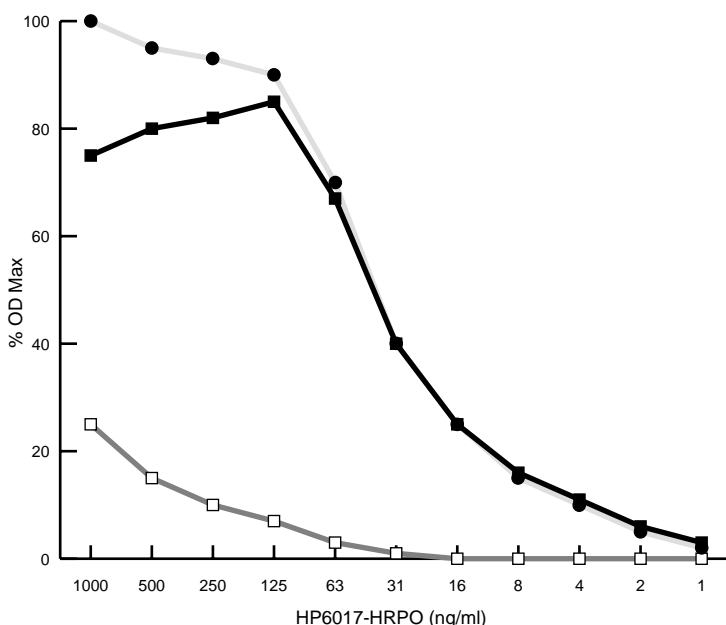
<b>IgG</b>	<b>Clinical Outcome of IgG deficiency</b>
IgG <sub>1</sub>	Hypo $\gamma$ -globulinemia Recurrent Infections Chronic Fatigue Syndrome (644) IgG <sub>3</sub> deficiency (645)
IgG <sub>2</sub>	Recurrent respiratory tract infections (646, 647) Deficiency of IgA and IgG <sub>4</sub> (648) Ataxia telanglectasia (623, 624) Chronic mucocutaneous candidiasis (649) Decreased response to bacterial infections (255) $\gamma$ -Interferon deficiency (650) Growth and psychomotor retardation (651) High incidence of colitis (652) High incidence of otitis media in children (653) Reduced levels following radiation therapy (642) Reduced levels in HIV-infected patients (251, 654)
IgG <sub>3</sub>	Recurrent infections Chronic lung disease Chronic mucocutaneous candidiasis (649) Wiskott-Aldrich syndrome (655)
IgG <sub>4</sub>	Recurrent respiratory tract infections Wiskott-Aldrich syndrome (655) Ataxia telanglectasia (624) High incidence of colitis (652) Reduced levels in HIV-infected patients (251, 654) Reduced levels following radiation therapy (642)

# VII. Appendices

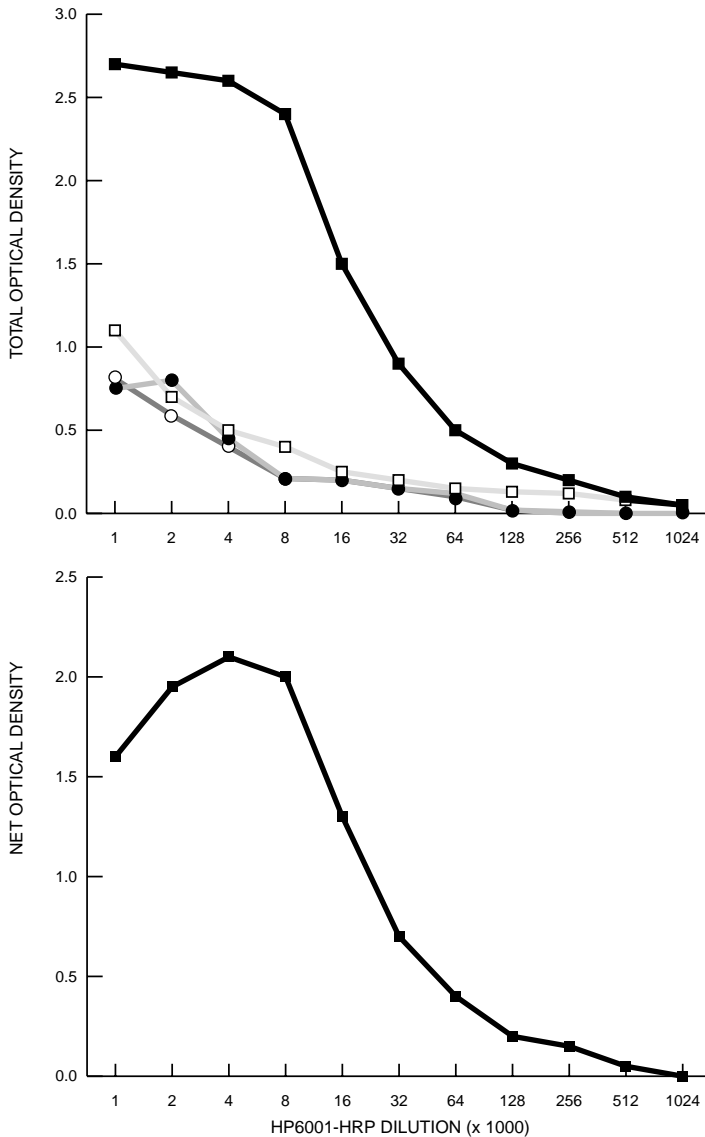
## A. Monoclonal Antibody Conjugates

Careful selection of the conjugated antibodies for detection of bound IgG is important to the quality of all immunoassays for IgG subclasses. Caution should be exercised when using antibodies from different species. Monoclonal antibodies to the human IgG subclasses can cross-react with immunoglobulins from mammalian species such as goat, sheep and rabbit (8). Use of an enzyme-conjugated antiserum to mouse IgG to detect mouse monoclonal antibody bound to a human IgG subclass can lead to undesirably high non-human IgG binding. The HP-series of monoclonal antibodies can be conjugated directly with horseradish peroxidase, alkaline phosphatase and biotin. Figure 12 shows an immunoreactivity and specificity analysis of peroxidase-conjugated antibody to human IgG Fc PAN (HP6017).

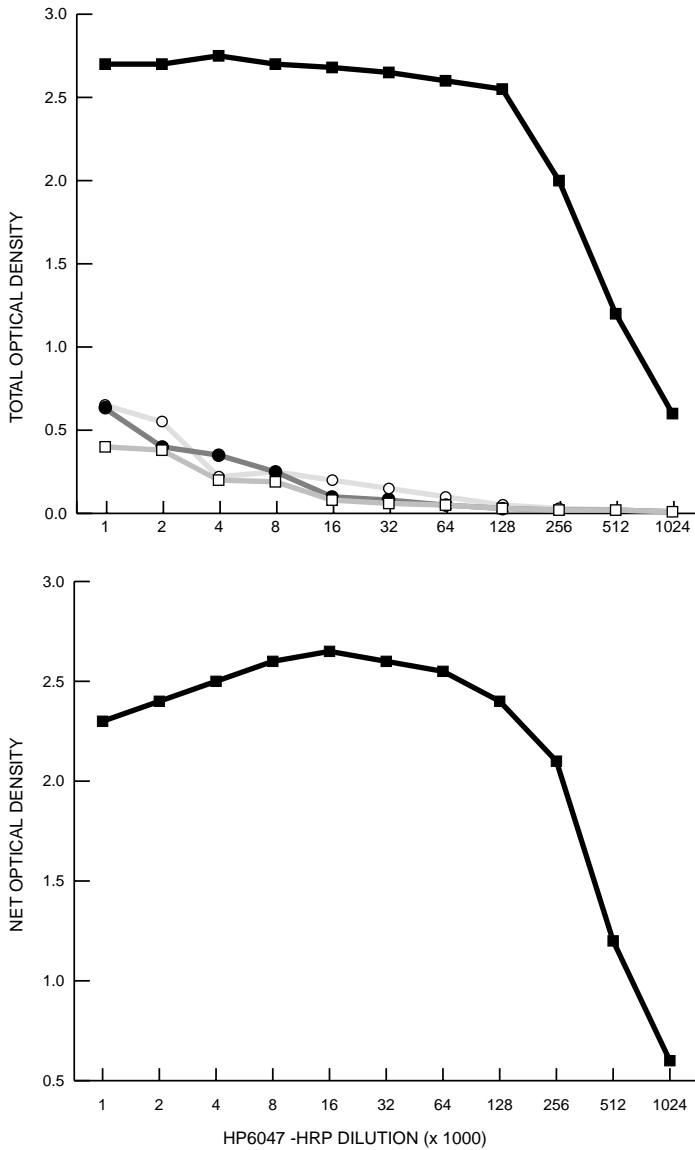
The IgG subclass specific antibodies (HP6001, HP6002, HP6047, and HP6023) all have been conjugated successfully with horseradish peroxidase and alkaline phosphatase. Figures 13–15 show dilutional analyses of peroxidase-conjugated HP6001, HP6047 and HP6023.



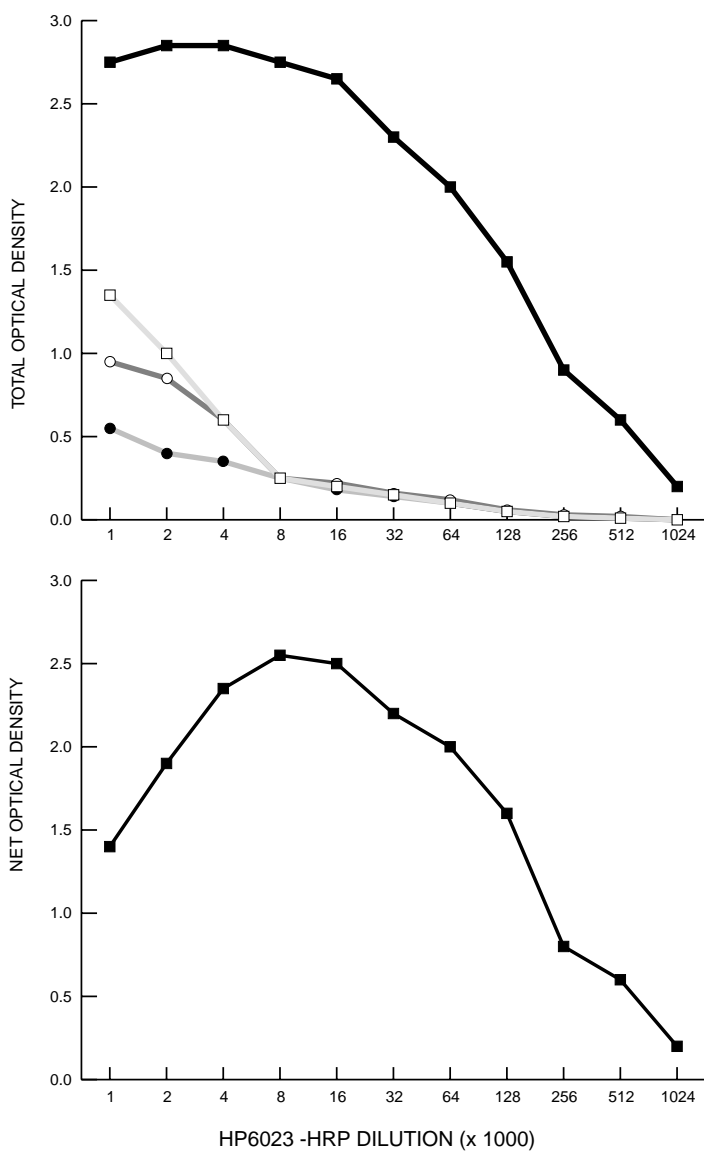
**Figure 12.** Analysis of immunoreactivity and specificity of horseradish peroxidase (HRP)-conjugated monoclonal mouse antibody to human IgG Fc (HP6017). Human (●) or mouse (□) IgG was coated onto microtiter plate wells (0.1 ml of 10 μg/ml of IgG in PBS, pH 7.4). The plates were blocked with PBS-0.1% BSA, and conjugated (HP6017) mouse antiserum to human Ig Fc (1000 to 1 ng/ml) was added (0.1 ml per well, diluted in PBS-0.1% BSA). After 2 hours at 37°C, plates were washed, o-phenylenediamine was added and the OD was measured at 495 nm. Nonspecific binding to mouse IgG (□) diminished to <5% at 125 ng/ml, while total binding to human IgG (●) remained high. Net binding of conjugated HP6017 (■) to human IgG was considered optimal at 125 ng/ml. Reproduced with permission from (116).



**Figure 13.** Dilutional analysis of horseradish peroxidase-conjugated anti-human IgG<sub>1</sub> Fc (HP6001). Human IgG<sub>1</sub> subclass myeloma (■), two control mouse monoclonal antibodies (HP6045, □; HP6046, ○) or BSA (●) were coated (0.1 ml, 10 µg/ml of IgG in PBS, pH 7.4, 37°C, 2 hours) on Immulon II microtiter plates. The plate was blocked with PBS, 0.5% BSA, and HRP-HP6001 (1:1000 to 1:1,024,000, 0.1 ml) was pipetted into the plate. After 2 hours at 37°C, the plate was washed, and bound peroxidase was detected with  $\alpha$ -phenylenediamine. Color development was stopped at 10 minutes with  $(\text{NH}_4)_2\text{SO}_4$ , and OD was read at 495 nm. Total OD is shown in the top panel for peroxidase-conjugated HP6001 binding to the four plate coatings. In the bottom panel, the net OD is presented (total OD – nonspecific OD in BSA-coated wells) as a function of serum dilution. The minimal HRP-HP6001 concentration that produced maximal net binding was 1:4000 to 1:8000.



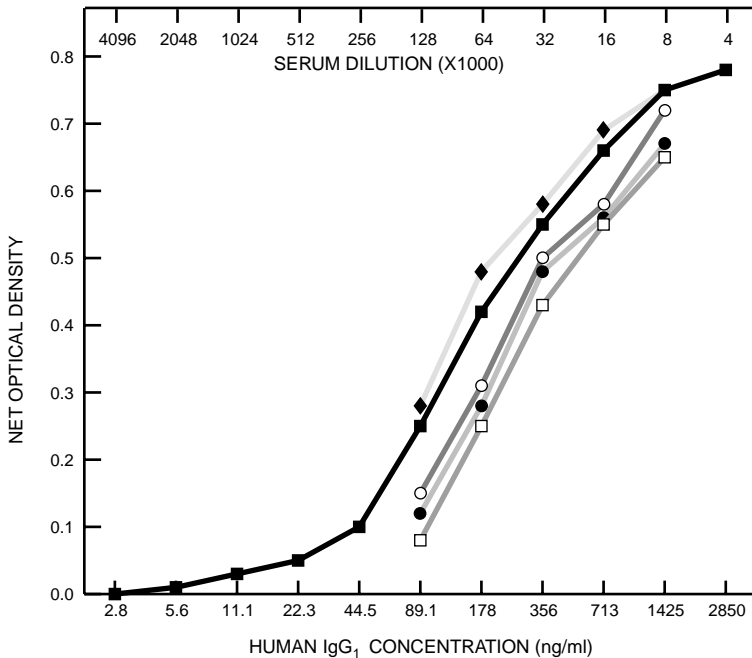
**Figure 14.** Dilutional analysis of horseradish peroxidase-conjugated antibody to human IgG<sub>3</sub> (HRP-HP6047). The experimental protocol described for Figure 13 was followed except that human IgG<sub>3</sub> myeloma (■) was used to coat plates. The minimal peroxidase-conjugated HP6047 concentration that produced maximal net binding was 1:32,000 to 1:64,000.



**Figure 15.** Dilutional analysis of horseradish peroxidase-conjugated antibody to human IgG<sub>4</sub> (HRP-HP6023). The experimental protocol described for Figure 13 was followed except that human IgG<sub>4</sub> myeloma (■) was used to coat plates. The minimal HRP-HP6023 concentration that produced maximal net binding was 1:8000 to 1:16,000.

## B. Quantitative IgG Subclass Immunoassay Protocol

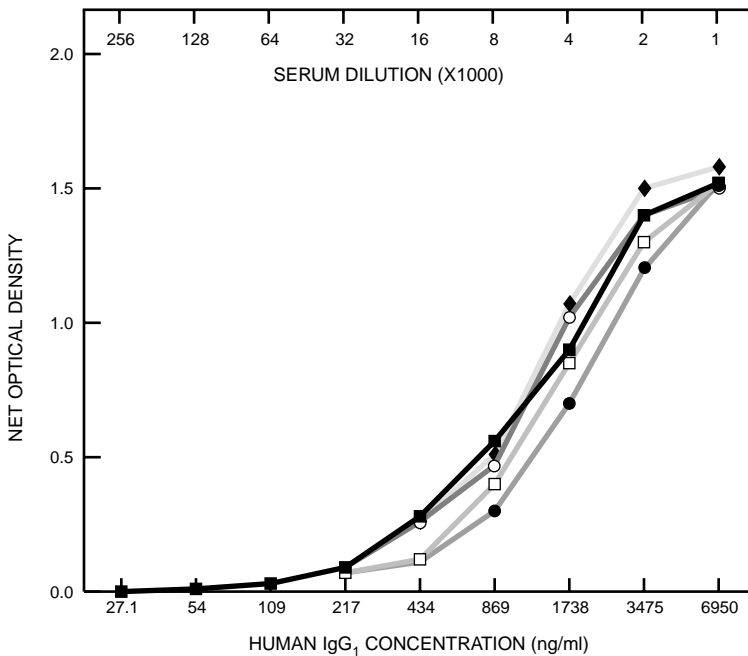
Several immunoassay configurations have been used to measure the quantity of each IgG subclass in human serum. The popular one uses specific antibody coupled to a solid phase (bead, plate) to extract IgG of a given subclass. Bound human IgG is detected by using a conjugated antibody to all human IgG subclasses (PAN). Performance of the HP-series monoclonal antibodies is shown by using this assay format in a two-site noncompetitive immunoenzymetric assay (IEMA). In general, monoclonal antibody is adsorbed to plastic microtiter plates (e.g., Immulon II, Dynatech, McClean, VA) by incubating 0.1 ml of antibody in PBS, pH 7.4 or 0.02 M carbonate buffer, pH 8.0 for 2 – 4 hours at 23 – 37°C or overnight at 4°C. The plates then are blocked with an irrelevant protein (e.g., PBS containing 0.5% BSA) for 1 hour at 23°C or 37°C. Serum (0.1 ml) is pipetted into replicate wells at a dilution greater than 1:1000 that has been made in PBS-BSA. After incubation (e.g., 2 – 4 hours at



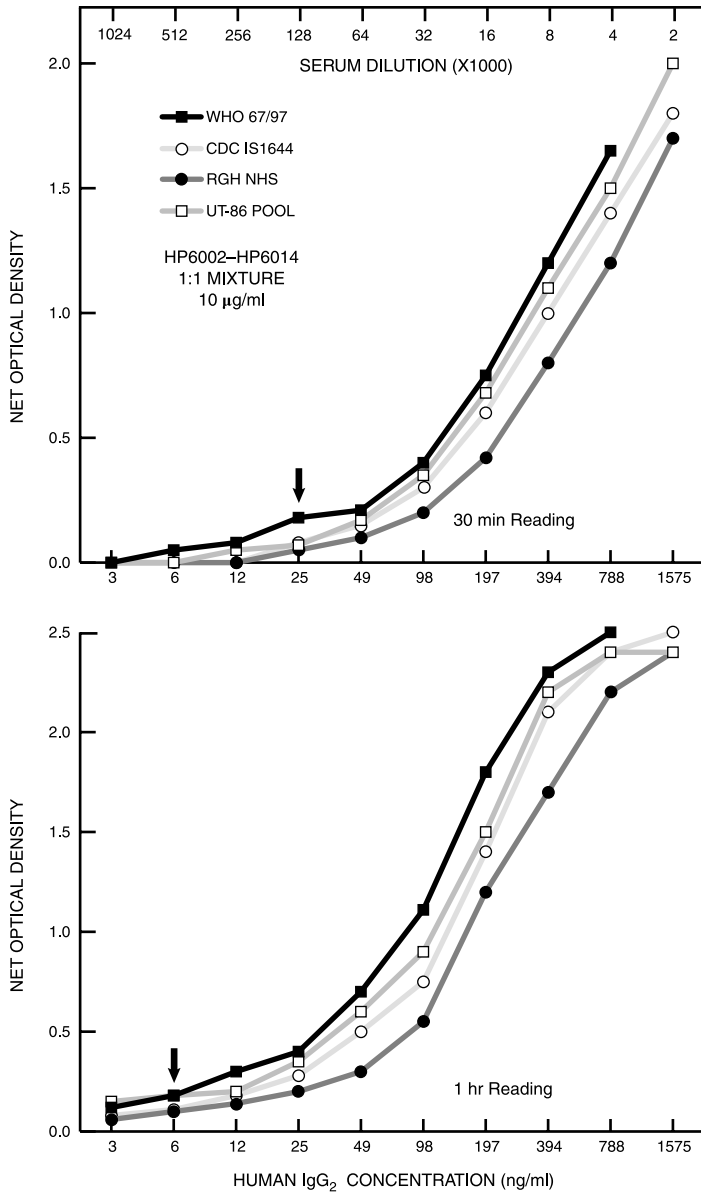
**Figure 16.** Dose-response curves generated in an IEMA of total serum IgG PAN by using the CDC reference serum (■) and test sera from healthy volunteers. Plates were coated at 10  $\mu$ g/ml overnight at 4°C with antibody to human IgG PAN Fd (1:1 mixture of HP6046 and HP6045). The plates were blocked and washed and serum was added. After a 2-hour incubation at 37°C, plates were rewashed and peroxidase-conjugated antibody to human IgG PAN (HP6017) was added (0.1 ml, 130 ng/ml). Incubation was performed for 2 hours at 37°C. Unbound conjugate was removed with buffer washes and 0.1 ml of OPD substrate (1 mg/ml in citrate buffer) was added. IgG levels in the test sera were interpolated from a reference curve prepared by using the CDC immunoglobulin standard (lower X axis). The test sera were analyzed at a dilution of 4,000 to 4,096,000 (upper X axis). Reproduced with permission (116).



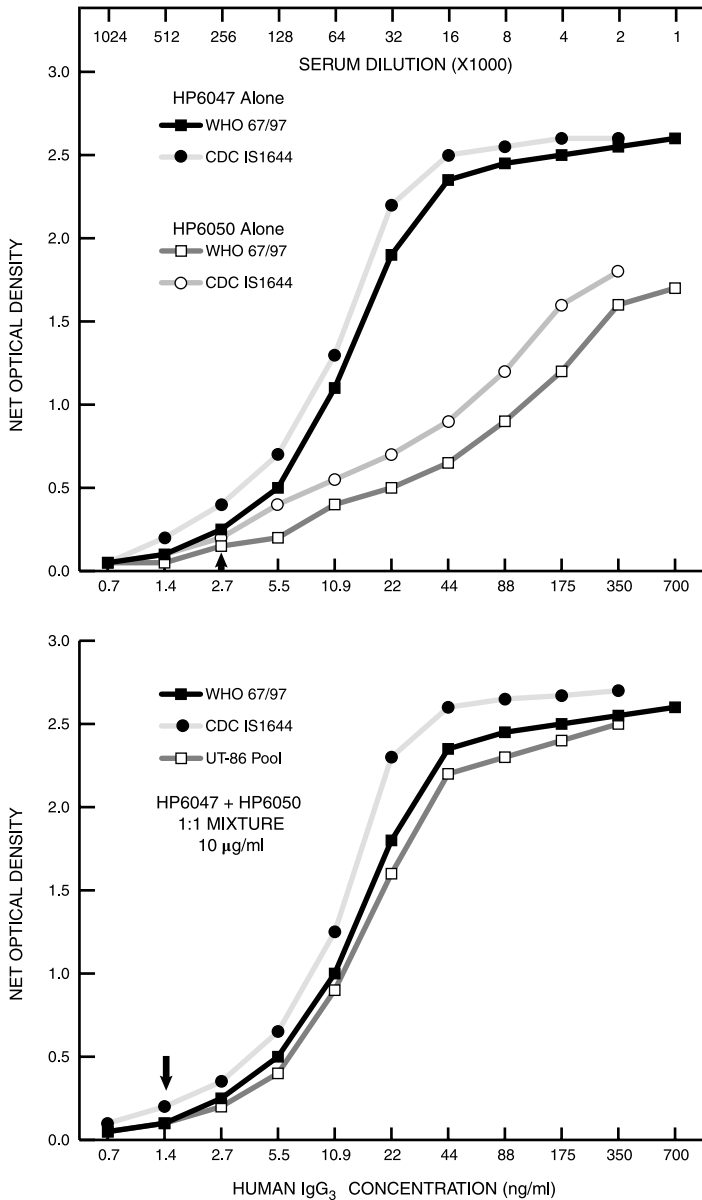
23 – 37°C), unbound serum proteins are removed with a buffer wash (e.g., PBS containing 0.05% TWEEN® 20), and enzyme-conjugated antibody to human IgG (absorbed against mouse IgG) is added. Several peroxidase- and phosphatase-conjugated antibodies to human IgG have been used; however, the mouse polyclonal or monoclonal (HP6017, HP6045, HP6046) antibodies to human IgG Fc have been particularly useful in the quantitative IgG subclass immunoassays. After the conjugate incubation (1 – 4 hours at 23 - 37°C), buffer washes are used to remove unbound conjugate, and the appropriate substrate is added (e.g., *o*-phenylenediamine for peroxidase, *p*-nitrophenylphosphate for alkaline phosphatase). Representative standard curves for the total, IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub> and IgG<sub>4</sub> assays are presented in Figures 16–20.



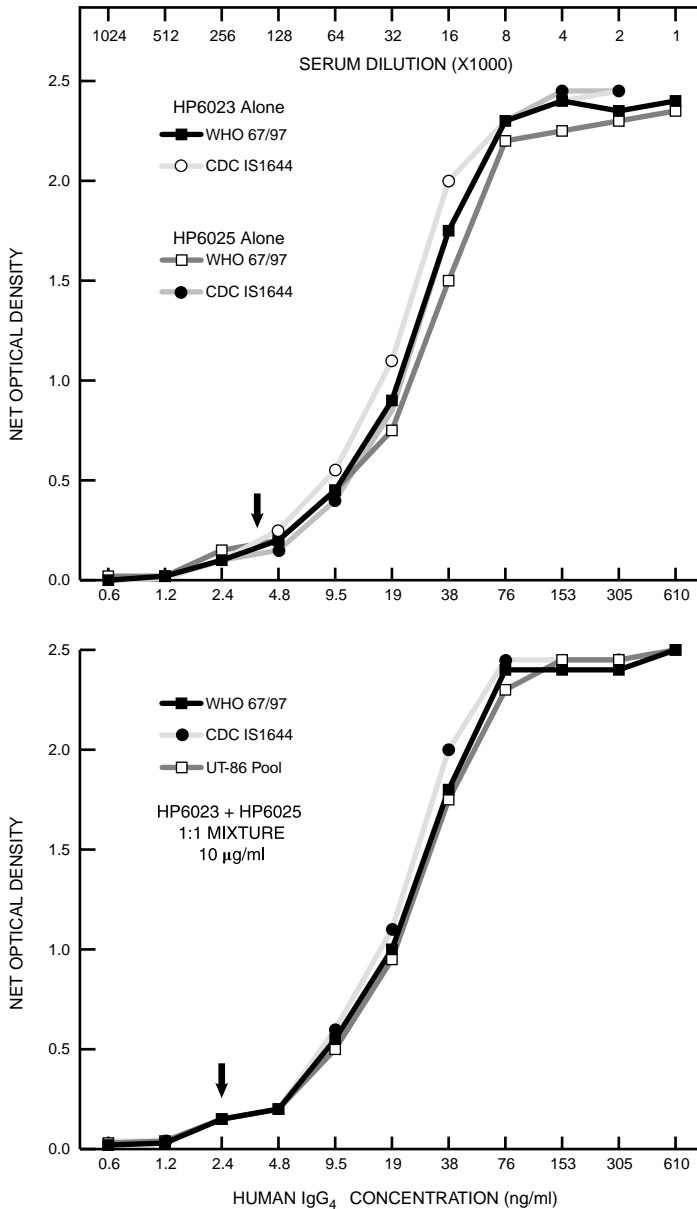
**Figure 17.** Dose-response curves generated in an IEMA of serum IgG<sub>1</sub> by using the CDC (■) and WHO (◆) reference sera. Microtiter plate wells were coated with antibody human IgG subclass 1 Fc (HP6001) at 10 mg/ml overnight, +4°C. The plates were blocked and washed and serum was added. After a 2-hour incubation at +37°C, plates were rewash ed, and alkaline phosphatase-conjugated polyclonal mouse anti-human IgG was added. After 2 hours at +37°C, unbound conjugate was removed with buffer washes, and substrate was added. Dilution curves of four human sera are parallel with the CDC Reference serum. Reproduced with permission (116).



**Figure 18.** Dose-response curves generated in an IEMA of serum IgG<sub>2</sub> by using the CDC and WHO reference sera and two human sera. Plates were coated with antibody to human IgG<sub>2</sub> (HP6002 + HP6014; 1:1 mixture) at 10 µg/ml, overnight at +4°C. The plates were processed as discussed for Figure 17. Parallelism of the dilution curves is demonstrable at the 30-minute and 1-hour readings. Arrows depict the IEMA's minimal detectable dose. Reproduced with permission from (116).



**Figure 19.** Dose-response curves generated in an IEMA of serum IgG<sub>3</sub> by using the CDC and WHO reference sera and a human serum pool. Plates were coated with antibody to human IgG<sub>3</sub> (HP6050 or HP6047 alone, top panel; HP6050 + HP6047; 1:1 mixture bottom panel) at 10 µg/ml, overnight at +4°C. The plates were processed as discussed for Figure 17. Assay sensitivity was enhanced by using the two antibodies in combination. Arrows depict the IEMA's minimal detectable dose. Reproduced with permission from (116).



**Figure 20.** Dose-response curves generated in an IEMA of serum IgG<sub>4</sub> by using the CDC and WHO reference sera and a human serum pool. Plates were coated with antibody to human IgG<sub>4</sub> (HP6023 or HP6025 alone, top panel; HP6023 + HP6025, 1:1 mixture, bottom panel) at 10 µg/ml, overnight at +4°C. The plates were processed as discussed for Figure 17. Assay sensitivity was enhanced by using the two antibodies in combination. Arrows depict the IEMA's minimal detectable dose. Reproduced with permission from (116).

# VIII. References

Please note that the following abbreviations have been used throughout the reference listing to conserve on space: Ag = antigen, Ab(s) = antibody(ies), MAb = monoclonal antibody, ANA = anti-nuclear antibody, SLE = systemic lupus erythematosus, Ig = immunoglobulin, SCID = severe combined immunodeficient

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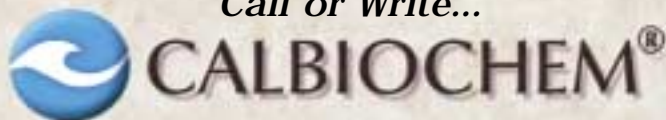
# IX. Anti-Human Antibodies and Conjugates

<b>Product</b>	<b>Cat. No.</b>
Anti-Human Ig, Light Chain, Type $\kappa$ , HP6062 (Mouse), Ascites	411600
Anti-Human Ig, Light Chain, Type $\lambda$ , HP6054 (Mouse), Ascites	411700
Anti-Human IgA, $\alpha$ -Chain Specific (Goat), Serum	401125
Anti-Human IgA, $\alpha$ -Chain Specific (Goat), Purified	401131
Anti-Human IgA, $\alpha$ -Chain Specific (Goat) Alkaline Phosphatase Conjugate	401132
Anti-Human IgA, $\alpha$ -Chain Specific (Goat) Fluorescein Conjugate	401134
Anti-Human IgA, $\alpha$ -Chain Specific (Goat) Peroxidase Conjugate	401135
Anti-Human IgA, Fab Fragment Specific, HP6123 (Mouse), Purified	411420
Anti-Human IgA, Fc Fragment Specific, HP6111 (Mouse), Purified	411421
Anti-Human IgA, Secretory, HP6130 (Mouse), Purified	411422
Anti-Human IgA, Secretory, HP6141 (Mouse), Purified	411423
Anti-Human IgE, $\epsilon$ -Chain Specific (Goat), Serum	411520
Anti-Human IgE, Fc Fragment Specific, HP6029 (Mouse), Ascites	411507
Anti-Human IgE, Fc Fragment Specific, HP6061 (Mouse), Ascites	411500
Anti-Human IgG, $\gamma$ -Chain Specific (Goat), Serum	401439
Anti-Human IgG, $\gamma$ -Chain Specific (Goat), Purified	401441
Anti-Human IgG, $\gamma$ -Chain Specific (Goat) Alkaline Phosphatase Conjugate	401442
Anti-Human IgG, $\gamma$ -Chain Specific (Goat) Fluorescein Conjugate	401444
Anti-Human IgG, $\gamma$ -Chain Specific (Goat) Peroxidase Conjugate	401445
Anti-Human IgG, $\gamma$ -Chain Specific (Rabbit) Alkaline Phosphatase Conjugate	401522
Anti-Human IgG, Fc Fragment Specific, HP6000 (Mouse), Ascites	411430
Anti-Human IgG, Fc Fragment Specific (Mouse) Alkaline Phosphatase Conjugate	411429
Anti-Human IgG, Fc Fragment Specific, HP6017 (Mouse), Ascites	411431
Anti-Human IgG, Fd Fragment Specific, HP6046 (Mouse), Ascites	411441
Anti-Human IgG, H & L Chain Specific (Goat), Purified	401954
Anti-Human IgG, H & L Chain Specific (Goat) Alkaline Phosphatase Conjugate	401453
Anti-Human IgG, H & L Chain Specific (Goat) Fluorescein Conjugate	401434
Anti-Human IgG, H & L Chain (Goat) Peroxidase Conjugate	401455
Anti-Human IgG <sub>1</sub> , IgG <sub>2</sub> , IgG <sub>3</sub> , HP6058 (Mouse), Purified	411428
Anti-Human IgG <sub>1</sub> , IgG <sub>3</sub> , IgG <sub>4</sub> , HP6019 (Mouse), Purified	411426
Anti-Human IgG <sub>2</sub> , IgG <sub>3</sub> , IgG <sub>4</sub> , HP6030 (Mouse), Purified	411427
Anti-Human IgG <sub>1</sub> , Fc Fragment Specific, HP6069 (Mouse), Ascites	411452
Anti-Human IgG <sub>1</sub> , Fc Fragment Specific, HP6069 (Mouse), Purified	411451
Anti-Human IgG <sub>1</sub> , Fc Fragment Specific, HP6070 (Mouse), Ascites	411454
Anti-Human IgG <sub>1</sub> , Fc Fragment Specific, HP6070 (Mouse), Purified	411455
Anti-Human IgG <sub>1</sub> , Fc Fragment Specific (Mouse) Alkaline Phosphatase Conjugate	401459
Anti-Human IgG <sub>1</sub> , Fc Fragment, HP6069 (Mouse) Biotin Conjugate	411543
Anti-Human IgG <sub>2</sub> , Fc Fragment Specific, HP6002 (Mouse), Ascites	411460
Anti-Human IgG <sub>2</sub> , Fc Fragment Specific, HP6002 (Mouse), Purified	411461
Anti-Human IgG <sub>2</sub> , Fc Fragment Specific (Mouse) Alkaline Phosphatase Conjugate	401462
Anti-Human IgG <sub>2</sub> , Fd Fragment Specific, HP6014 (Mouse), Purified	411466



<b>Product</b>	<b>Cat. No.</b>
Anti-Human IgG <sub>2</sub> , Fc Fragment (Mouse) Biotin Conjugate	411462
Anti-Human IgG <sub>3</sub> , Hinge Specific, HP6047 (Mouse), Ascites	411480
Anti-Human IgG <sub>3</sub> , Hinge Specific, HP6047 (Mouse), Purified	411482
Anti-Human IgG <sub>3</sub> , Hinge Specific, HP6050 (Mouse), Ascites	411481
Anti-Human IgG <sub>3</sub> , Hinge Specific, HP6050 (Mouse), Purified	411484
Anti-Human IgG <sub>3</sub> , Fc Fragment Specific (Mouse) Alkaline Phosphatase Conjugate	401465
Anti-Human IgG <sub>3</sub> , Hinge, HP6047 (Mouse) Biotin Conjugate	411483
Anti-Human IgG <sub>4</sub> , Fc Fragment Specific, HP6023 (Mouse), Ascites	411490
Anti-Human IgG <sub>4</sub> , Fc Fragment Specific, HP6025 (Mouse), Purified	411492
Anti-Human IgG <sub>4</sub> , Fc Fragment Specific (Mouse) Alkaline Phosphatase Conjugate	401468
Anti-Human IgG <sub>4</sub> , Fc Fragment, HP6023 (Mouse) Biotin Conjugate	411493
Anti-Human IgM, Fc Fragment Specific, HP6081 (Mouse), Ascites	411802
Anti-Human IgM, Fc Fragment Specific, HP6083 (Mouse), Ascites	411804
Anti-Human IgM, Fc Fragment Specific, HP6086 (Mouse), Ascites	411800
Anti-Human IgM, $\mu$ -Chain Specific (Goat), Serum	401900
Anti-Human IgM, $\mu$ -Chain Specific (Rabbit), Purified	401852
Anti-Human IgM, $\mu$ -Chain Specific (Goat) Alkaline Phosphatase Conjugate	401902
Anti-Human IgM, $\mu$ -Chain Specific (Mouse) Alkaline Phosphatase Conjugate	401909
Anti-Human IgM, $\mu$ -Chain Specific (Goat) Fluorescein Conjugate	401904
Anti-Human IgM, $\mu$ -Chain (Goat) Peroxidase Conjugate	401905

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