Recombinant Mouse-Human Chimeric Antibodies as Calibrators in Immunoassays That Measure Antibodies to Toxoplasma gondii
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In the present study, we examined the feasibility of using recombinant antibodies containing murine variable regions and human constant regions as calibrators or controls in immunoassays. As a model system, we chose the Abbott IMx Toxo immunoglobulin M (IgM) and Toxo IgG assays designed to detect antibodies to Toxoplasma gondii. Two mouse monoclonal antibodies were selected based on their reactivity to the T. gondii antigens P30 and P66. Heavy- and light-chain variable-region genes were cloned from both hybridomas and transferred into immunoglobulin expression vectors containing human kappa and IgG1 or IgM constant regions. The constructs were stably transfected into Sp2/0-Ag14 cells. In the IMx Toxo IgG assay, immuno-reactivity of the anti-P30 chimeric IgG1 antibody paralleled that of the positive human plasma-derived assay calibrators. Signal generated with the anti-P66 chimeric IgG1 antibody was observed to plateau below the maximal reactivity observed for the assay calibrator. Examination of the IgM chimeric antibodies in the IMx Toxo IgM assay revealed that both the anti-P30 and anti-P66 antibodies matched the assay index calibrator manufactured with human Toxo IgM-positive plasma. When evaluated with patient samples, the correlation between results obtained with the chimeric antibody calibrators and the positive human plasma calibrators was \( r^2 = 0.985 \). These data demonstrate that chimeric mouse-human antibodies are a viable alternative to high-titer positive human plasma for the manufacture of calibrators and controls for diagnostic immunoassays.

Serological immunoassays designed to detect specific antibody present in patient samples provide a rapid and sensitive method to monitor for infectious agents, allergy, and autoimmunity (2). Diagnostic immunoassays and kits designed to measure antibodies typically include one or more components containing the specific antibody being measured that function as calibrators (standards) and/or a positive control (3). Calibrators are used to establish calibration curves in quantitative assays for interpolation of antibody concentration in the patient sample, or alternatively, a single index calibrator may be used to establish the assay cutoff in a qualitative assay. The positive control is used to establish assay performance characteristics and is a useful indicator of the integrity of the reagents. Calibrators and positive controls are usually prepared by spiking known quantities of specific antibody derived from seropositive plasma or serum into the negative control reagent.

However, the use of plasma or serum has several significant drawbacks, including increasing difficulty in sourcing large volumes with high titer and specificity, lot-to-lot variability in immunoglobulin G (IgG) or IgM specificity and affinity, limitations with respect to characterization, and cost.

An alternative method for the manufacture of calibrators and controls that circumvents the use of human plasma or serum would represent a significant advance. Hybridoma technology (12) offers an indefinite supply of monoclonal antibodies; however, murine antibodies do not react in the assay format required to measure specific human IgG or IgM and therefore cannot be used to standardize assays to measure human antibodies. With the development of recombinant DNA technology, it has become possible to combine the heavy (H)- and light (L)-chain variable (V) regions of a desired mouse monoclonal antibody with human constant (C) regions, creating hybrid (chimeric) antibody molecules (1, 15). Chimeric antibodies can be reproducibly generated in virtually unlimited quantities and are homogeneous in specificity and affinity.

In the present study, we developed mouse-human chimeric IgM and IgG1 antibodies specific for Toxoplasma gondii and evaluated their performance as calibrators in the Abbott IMx Toxo IgM and IMx Toxo IgG assays (13, 16).

MATERIALS AND METHODS

Cell lines. The hybridomas 1-706-139 (H1) and 5-465-210 (H5) were established by fusion of immunized Swiss Webster spleen cells to Sp2/0-Ag14 cells (Abbott Laboratories, Abbott Park, Ill.; American Type Culture Collection, Rockville, Md.).

Isolation of immunoglobulin V regions. PCR cloning of immunoglobulin V regions was performed as previously described (6) with primers designed by Jones and Bendig (9) with altered restriction sites (Table 1). M-IgG2b and M-IgG2a were used for specific cDNA synthesis of heavy-chain V regions from H1 and H5, respectively. MK-REV was used for cDNA priming of kappa (k) light-chain variable regions. H1 V\(_k\) and V\(_k\) regions were amplified with primer combinations MHV-9/M-IgG2a and MKV-1/MK-REV, respectively. For H5, V\(_k\) and V\(_k\) regions were amplified with MHV-9/M-IgG2a and MKV-2/MK-REV, respectively. Amplifications consisted of 30 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 1 min. PCR-derived products were isolated by the Promega (Madison, Wis.) Magic PCR Peps DNA purification system, digested (SalI and BglII), and cloned into pUC18 (BRL Life Technologies, Gaithersburg, Md.).

Chimeric IgM expression constructs. The immunoglobulin expression vector pDH2L2 (5) (Fig. 1A) was modified by replacing the human IgG1 constant region (C\(_\text{H}\)1) with a human IgM constant region (C\(_\text{H}\)M). First, the oligonucleotides 5′ GGAACTAGTGGAGC 3′ and 5′ TCCACATGTCGCC 3′ were annealed and cloned into the SacII site in pDH2L2 (upstream of C\(_\text{L}\)) to introduce a SpeI site. Next, the secretory portion of the C\(_\text{L}\) gene (membrane exons excluded) was PCR-
amplified by using 1 ng of a plasmid containing a genomic clone of human C\_\mu (pN-\mu LNP DNA; provided by Marc Shulman, University of Toronto, Toronto, Ontario, Canada) as template. 50 pmol each of primers A (Table 1) (Promega) and B (EcoRV cloning site), and 24 cycles of 94°C for 30 s, 63°C for 30 s, and 72°C for 90 s. Gel-isolated C\_\mu cloning site) and B (SpeI cloning site), and 24 cycles of 94°C for 30 s, 63°C for 30 s, and 72°C for 90 s. Gel-isolated C\_\mu product (spanning from 128 nucleotides [nt] upstream of C\_\mu exon to 152 nt downstream of the polyadenylation signal after C\_\mu [positions 17 to 2278 in reference 20]) was ligated into pGEM-T (Promega) and sequenced. Firstly, the SpeI-EcoRV fragment containing C\_\mu was excised and cloned into SpeI-PvuII digested pHIL2, replacing C\_\mu.

Primers used for PCR-mediated transfer of V\_\mu and V\_\nu are shown in Table 1. The H\_\nu V\_\nu gene fragments were amplified with primer combinations VK1-3'/VH1-3' and VH5-3'/VH5-3', respectively. The 100-μl volume reaction mixtures included 50 pmol each of primer and 1 ng of genomic DNA containing a V-region gene (see above). Amplification consisted of 22 cycles of 94°C for 30 s, 62°C for 30 s, and 72°C for 1 min. V\_\nu product was digested with XbaI-BamHI and cloned into the vector. Subsequently, V\_\nu product was introduced into the V\_\nu-containing vector by using Xhol-HindIII, generating pHIL2-24-9SB1 (Fig. 1B).

The H\_\nu V\_\nu gene transfer was performed in the same manner by using primer combinations VK5-3'/VH1-3' and VH5-3'/VH5-3', respectively, yielding pHIL3-19-95A.

Transfer of V regions into the IgG1 expression vector pdHL2. An XbaI-HindIII fragment containing both V\_\nu and V\_\nu was excised from each of the IgM vectors and introduced into XbaI-HindIII digested pHIL2, yielding pHIL9-14-94/4.1 (H1 set) and pHIL2-5/465 (H5 set).

Sequencing. The plasmid template for sequencing was prepared with Magic Sequencing. The plasmid template for sequencing was prepared with Magic Sequencing.

Restriction endonuclease sites are underlined.

Transfection of murine myeloma cells. Sp2/0-Ag14 cells were transfected by electroporation as previously described (6). IgM constructs were transfected by using a modified version of the protoplast fusion technique (19). Briefly, Sp2/0-Ag14 cells were resuspended in the protoplast suspension, transferred to a 60-mm-diameter dish, and centrifuged at 650 × g for 8 min. The supernatant was aspirated, and 1.5 ml of 50% (wt/vol) polyethylene glycol (Sigma Hybri-Max) in phosphate-buffered saline (PBS) warmed to 37°C was added. The dish was centrifuged at 110 × g until 90 s had elapsed after polyethylene glycol addition. The cells were resuspended by pipetting in two 5-ml volumes and one 10-ml volume of warmed wash solution (Dulbecco Modified Eagle medium [DMEM] supplemented with 1% fetal bovine serum, 50 U of penicillin per ml, and 50 μg of streptomycin per ml), which were added to a 50-ml centrifuge tube containing 15 ml of the wash solution. After centrifugation at 225 × g for 7.5 min, the cells were resuspended in plating medium (DMEM supplemented with 10% fetal bovine serum, 50 U of penicillin per ml, 50 μg of streptomycin per ml, and 100 μg of kanamycin per ml [Sigma]), plated in one 96-well dish, and incubated at 37°C. After 24 h (day 1), 100 μl of selective medium (plating medium supplemented with 0.1 μm methotrexate [MTX, Adria Laboratories, Columbus, Ohio]) was added. On day 5, 50 μl of the selective medium was added per well. After 2 days, 100 μl was removed from each well and 100 μl of fresh selective medium was added per well. MTX-resistant colonies were tested for secretion of chimeric antibody by an enzyme-linked immunosorbent assay (ELISA). Transfectants secreting chimeric antibody were cloned by limiting dilution and passages in medium containing 0.1 μM MTX.

Assays for chimeric antibody production. Transfectants were assayed by ELISA for production of chimeric IgG1 as previously described (6). To assay for chimeric IgM, plates were coated with 0.36 μg of goat anti-human IgM (Fc\_\gamma-specific; Jackson ImmunoResearch, West Grove, Pa.) per ml in Dulbecco's phosphate-buffered saline (D-PBS). Peroxidase-labeled goat anti-human IgM (Fc\_\gamma; Jackson ImmunoResearch) at 0.8 μg/ml was used as the enzyme antibody conjugate, with ChromPure human IgM (Jackson ImmunoResearch) as a standard.

FIG. 1. Schematic diagram of the IgG1 expression vector pdHL2 (A) and anti-P30 IgM construct pHIL2-24-95B1 (B). The locations of the genomic C\_\mu, C\_\nu, and C\_\lambda genes are indicated. Abbreviations: E\_\mu, immunoglobulin H-chain enhancer; P, the metallothionein promoter; pA, polyadenylation signal sequence; ori, pBR322 origin of replication; AMBl, β-lactamase gene; DFR, dihydrofolate reductase gene. The positions of V\_\mu and V\_\nu cassettes are shown in panel B. Abbreviations of unique restriction sites: B, BanII; H, HindIII; P, PvuII; S, SalI; Sa, SlaI; Sp, SpeI; Xb, XbaI; Xh, XhoI.
Antibody concentrations were determined by radial immunodiffusion (RID). Seven- to 14-day cell culture supernatants were adsorbed, and then 5- and 10-μl samples for IgG and IgM, respectively, were applied to each well of human IgG or human IgM RID plates (The Binding Site, San Diego, Calif.). Standards (25, 50, 75, 100, and 150 μg/ml) were run on each plate. The IgM and IgG1 standards were ChromPure human IgM and protein A-purified anti-P66 IgG, respectively. Plates were incubated in an inverted position at 35 to 37°C for 16 to 22 h. Ring diameter was determined by using a calibrated RID electronic plate reader (The Binding Site).

Purification of chimeric antibodies. To purify chimeric IgG1 antibodies, culture supernatants were dialyzed overnight against 0.1 M sodium phosphate (pH 8.2) with 0.1% sodium azide, filtered (0.2-μm-pore-size filter), and passed through a column containing PerSepTive Biosystems (Cambridge, Mass.) Poros 50A resin by using a Bio-Rad low-pressure chromatography system. Antibody was eluted with 0.1 M citrate–0.15 M NaCl (pH 3.0), and pooled fractions were dialyzed against PBS (10 mM sodium phosphate, 150 mM NaCl) (pH 7.2).

To purify chimeric IgM antibodies, culture supernatants were dialyzed 1:1 with H2O and loaded on a column containing 175 ml of PBS-equilibrated DEAE FastFlow resin (Pharmacia). Antibody was eluted with 10 mM sodium phosphate–300 mM NaCl at pH 7.2.

IMx evaluation of chimeric calibrators. Reactivity of chimeric IgG1 and IgM antibodies was measured with the Abbott IMx Toxo IgG 2.0 antibody assay (Abbott Laboratories) and the Abbott IMx Toxo IgM 2.0 antibody assay, respectively. Testing was done as described in the assay package insert, except where stated.

Antibody concentrations were determined by radial immunodiffusion (RID). To purify chimeric IgM antibodies, culture supernatants were dialyzed 1:1 with H2O and loaded on a column containing 175 ml of PBS-equilibrated DEAE FastFlow resin (Pharmacia). Antibody was eluted with 10 mM sodium phosphate–300 mM NaCl at pH 7.2.

Nucleotide sequence accession numbers. GenBank accession numbers for V region sequences are as follows: for 1-706-139 VH, AF031633; for 5-465-210 VH, AF031634; and for 5-665-210 VH, AF031636.
To produce chimeric mouse-human IgG1, the V gene cassettes were cloned into the immunoglobulin expression vector pdHL2 (Fig. 1A). This vector contains genomic clones of the human kappa (Ck) and IgG1 (Cκ1) C-region genes. Both loci are controlled by a metallothionein I promoter and a mouse immunoglobulin H-chain enhancer. An altered dihydrofolate reductase gene serves as a selectable marker. Introduction of the murine Vκ and Vλ regions results in expression of chimeric mouse-human IgG1 antibody.

To produce chimeric mouse-human IgM antibodies, pdHL2 was modified by replacement of the Cκ1 gene with a genomic clone of the human IgM (Cκm) gene. Sequence analysis of the Cκm clone revealed only a single base change relative to the published germ line sequence (20), the insertion of a T between residues 1583 and 1584 in the Cκm3-Cκm4 intron. This alteration would not be expected to affect expression of the Cκm gene since it is in the intron. The H1 and H5 VH and VH cassettes were cloned into the IgM expression vector to generate chimeric IgM antibody specific for P66 and P30, respectively.

Expression of IgM and IgG chimeric antibodies. The chimeric antibody constructs were transfected by electroporation into Sp2/0-Ag14 cells. Multiple MTX-resistant colonies from each transfection were screened for production of chimeric antibody by using an anti-human antibody ELISA. Transfectants that tested positive were expanded and cloned. Clones secreting high levels of chimeric antibody were further subcloned. The observed production levels for the anti-P30 and anti-P66 chimeric IgG1 antibodies were 90 μg/ml or greater. In contrast, IgM transfectants established by electroporation secreted very low levels of antibody. Prototrans fusion is an alternative method for transfection (1, 19) that often results in introduction of higher copy numbers of vector. The IgM constructs were introduced into Sp2/0-Ag14 cells by protoplast fusion in an effort to enhance expression. A clone secreting anti-P30 chimeric IgM antibody that produces 115 μg/ml was isolated. Transfection of the anti-P66 chimeric IgM construct met with more limited success. Transfectants secreting chimeric IgM were obtained, but secretion levels were low (<1 μg/ml). In all but one case, production of recombinant chimeric antibody by the transfectants greatly exceeded that of the hybridomas from which they were derived. Analysis of the IgM chimeric antibody revealed that it is pentameric.

Assay performance of the chimeric antibodies. Purified chimeric IgG1 and IgM antibodies were examined for immuno-reactivity to T. gondii in the IMx Toxo IgG and IMx Toxo IgM assays. These assays utilize microparticles coated with formalin-treated T. gondii as the solid phase to capture anti-human IgG or IgM antibody-labeled conjugates. Reactivity of purified anti-P30 chimeric IgG1 was compared to the reactivity of human anti-T. gondii antibody in the IMx Toxo IgG assay. The calibrators used in this assay are manufactured from pooled anti-T. gondii IgG-positive plasma and are matched to the World Health Organization International Standard for anti-T. gondii antibody at the following levels: 0, 10, 50, 100, 200, and 300 IU/ml. The F calibrator (300 IU/ml) marks the upper end of the dynamic range of the assay. The anti-P30 chimeric IgG1 antibody at 218 μg/ml was found to give the same signal as the F calibrator in the IMx assay. Twofold serial dilutions of the anti-P30 chimeric IgG1 antibody into negative human serum were tested in duplicate. The observed calibration curve for the chimeric IgG1 antibody was similar to the dilution profile of the human plasma-derived assay calibrator (Fig. 4A). At a concentration of 870 μg/ml, the...
anti-P30 chimeric IgG1 antibody generated a signal of 4,261 cps/s in the assay. The dilution curve for the anti-P66 chimeric IgG1 antibody, compared to the human plasma-derived assay calibration curve, is shown in Fig. 4B. The starting concentration of the purified anti-P66 chimeric IgG1 antibody was 1.27 mg/ml, and a signal of only 1,600 cps/s was achieved. The signal begins to plateau at antibody concentrations between 300 and 600 µg/ml and never reaches the level of the F calibrator (300 IU/ml). Concentration of the antibody to 11.59 mg/ml did not result in a significant increase in the IMx assay signal generated.

The IMx Toxo IgM assay differs from the IMx Toxo IgG assay in that it is a qualitative assay. The purpose of the calibrator in the IgM assay is to establish the assay cutoff based on the rate value generated with a consistent concentration of anti-*T. gondii* IgM antibodies. Purified anti-P30 chimeric IgM and anti-P66 chimeric IgM antibodies were diluted by twofold serial dilutions into negative human plasma, and the resulting values were compared to that of the IMx Toxo IgM index calibrator. The anti-P30 chimeric IgM and anti-P66 chimeric IgM antibodies gave signals equivalent to that of the index calibrator at concentrations of 13 and 10 µg/ml, respectively (Fig. 5).

**Evaluation of patient samples by using kits calibrated with chimeric antibody-derived calibrators.** The anti-P30 chimeric IgG1 and IgM antibodies were diluted to match human plasma-derived calibrators to demonstrate the utility of the chimeric antibody as a source of calibrators for the IMx Toxo IgG and IMx Toxo IgM assays. Human plasma-derived calibrators for the IMx Toxo IgG assay ranged from 0 to 300 IU/ml. The anti-P30 chimeric IgG1 antibody was diluted in negative human plasma to match each level of the human plasma calibrators within 2% of IMx assay calibrator rates. Calibration curves obtained for each of the kit calibrators and anti-P30 chimeric IgG1-derived calibrators were similar (Fig. 6A). Thirty-six serum specimens with anti-*Toxoplasma* IgG concentrations less than 60 IU/ml were evaluated in the IMx Toxo IgG assay using the chimeric antibody calibrators as the source of the standard curve, and the results were compared with those of the same specimens run in the assay using human plasma-derived calibrators. The correlation exhibited an r^2 value of 0.985 and a slope of 0.991 (Fig. 6B).

The anti-P30 chimeric IgM antibody was diluted in negative human plasma to match within 2% the rate level of the human plasma IgM index calibrator used in the IMx Toxo IgM assay. The specimen results are expressed as index values relative to the index calibrator. Specimens with index values equal to or greater than 0.600 are considered reactive for IgM antibody to *T. gondii*. Thirty-one serum specimens were evaluated in the IMx Toxo IgM assay using the anti-P30 chimeric antibody.
index calibrator, and the results were compared with those of
the same specimens run in the assay using human plasma-
derived calibrators. The correlation exhibited an $r^2$ value of
0.999 and a slope of 1.039 (Fig. 7).

DISCUSSION

In the present report, we describe the development and
utility of mouse-human chimeric antibodies as calibrating re-
agents in automated immunoassays measuring human antibod-
ies to *T. gondii*. Murine monoclonal antibodies specific for P30
(11) and P66 (18) were selected for conversion to mouse-

human chimeric antibodies. Functional immunoglobulin V re-

FIG. 6. Comparison of IgG1 chimeric calibrator to human plasma-derived calibrator in the IMx Toxo IgG assay. (A) Calibration curve generated with IMx Toxo
IgG kit calibrators compared to curve of anti-P30 chimeric IgG1 antibody calibrators matched to each level; (B) specimen correlation comparing calibration with
anti-P30 chimeric IgG1 calibrators to calibration with the kit human plasma calibrators ($n = 36; r^2, 0.985; \text{slope, 0.991}$).

FIG. 7. Comparison of IgM chimeric calibrator to human plasma-derived calibrator in the IMx Toxo IgM assay. Shown is specimen correlation comparing
 calibration with the anti-P30 chimeric IgM index calibrator to calibration with the IMx Toxo IgM assay human plasma-derived index calibrator ($n = 31; r^2,
0.999; \text{slope, 1.039}$).
gondii-reactive human plasma, it would be expected to contain antibodies against many different T. gondii proteins (17, 18) and to multiple epitopes on each antigen. Thus, the observation that the signal of a monoclonal reagent specific for one epitope of a single T. gondii protein is unable to match the cumulative signal generated by the polyclonal calibrator reagent is not necessarily unexpected. It is conceivable that blending of two or more monoclonal reagents may better mimic the polyclonal human antibody calibrator and more closely approximate the routine human test samples. In preliminary tests where the anti-P66 IgG1 chimeric antibody was combined with the anti-P30 chimeric IgG1, a signal exceeding the high end of the dynamic range (F calibrator) was achievable and the dilution profile was similar to that of the human plasma-derived Toxo IgG calibrator (data not shown). The concept of utilizing more than one chimeric antibody to generate a calibrator might become particularly important if one is measuring a response to more than one epitope of an antigen or to more than one antigen when considering issues such as the differential stability of antigen reagents in immunoassay kits. If the epitope recognized by one of the chimeric antibodies was impaired, a second epitope may be required to accurately calibrate the assay. Determination of whether it is advantageous to pool more than one chimeric antibody reagent will be dependent upon the desired performance characteristics for the calibrator and can be determined empirically.

As opposed to the quantitative Toxo IgG assay, the Toxo IgM assay is a qualitative assay that utilizes a single index calibrator to determine the cutoff value. When examined in the Toxo IgM assay, acceptable calibration curves were obtained independently for both the anti-P30 and anti-P66 IgM chimeras. The feasibility of using a chimeric antibody calibrator was examined by comparing the results obtained for 31 T. gondii-reactive specimens in the Toxo IgM assay to those obtained by using the anti-P30 chimeric IgM index calibrator. There was a high correlation between the results generated with the human plasma-derived and chimeric IgM antibody calibrators. These data demonstrate that the anti-P30 IgM chimeric antibody can be substituted for T. gondii-reactive human plasma in the manufacture of an index calibrator.

An advantage of chimeric antibody technology in the manufacture of calibrating reagents is its flexibility. One can readily screen candidate monoclonal antibodies for desired properties such as specificity and affinity prior to the more labor-intensive steps of cloning the V regions and production of chimeric antibody. Binding properties of the chimeric antibody should reflect those of the original monoclonal antibody. In the present study, the choice of antibody specificity was based on competitive inhibition studies using T. gondii-reactive human plasma in an effort to identify monoclonal antibodies reacting with immunodominant epitopes. Presumably, the more closely the epitope reactivity of the chimeric antibodies and human test samples are matched, the better the calibrator. In some cases, this may require the use of more than one chimeric antibody recognizing different antigens or of multiple epitopes on the same antigen. Ideally, the affinity of the chimeric antibodies would be similar to that of the antibodies being monitored in the test samples. Other factors such as epitope stability (i.e., after heat stress or prolonged storage) can also be examined prior to conversion of the monoclonal antibody to the chimeric format.

In addition to epitope specificity, proper selection of heavy-chain isotype is also important. In most cases, immunoassays that monitor specific antibody are designed to detect antibody classes or subclasses with in vivo or diagnostic relevance. In patients acutely infected with T. gondii, IgM is the predominant class of antibody, whereas in patients with acquired or reactivated toxoplasmosis, IgG1, IgG2, IgG3, and IgA antibodies can be detected (4). The IMx Toxo IgG assay uses a polyclonal anti-human IgG as the conjugate reagent. We found that the chimeric IgG1 antibody calibrates the IMx assay accurately over multiple lots of the polyclonal anti-human IgG reagent (data not shown).

The use of chimeric antibodies in preparation of calibrators and positive controls for immunoassays offers several substantial advantages over traditional methods utilizing high-titer plasma or serum, as well as other alternatives such as chemical coupling of human IgG Fc’ fragments to a murine monoclonal antibody (14). First, the chimeric antibody can be produced continuously with the same affinity and specificity. Reduction in lot-to-lot variability over time with respect to antibody class composition, titer, specificity, and affinity would be expected to yield a consistent calibrator that should give the most reproducible patient results. Second, virtually unlimited quantities of the chimeric antibodies can be generated. Recombinant cell lines can stably produce chimeric antibodies at manufacturable levels and at a reasonable cost. Finally, the homogeneous nature of the chimeric antibodies allows for better characterization.

Mouse-human chimeric antibodies have previously been shown to be useful as quality control reagents and for quantitation of specific antibody in reference standards by heterologous interpolation (7, 8). The present study establishes the feasibility of using mouse-human chimeric antibodies as calibrator reagents in automated immunoassays for the measurement of human antibodies specific for T. gondii. This is the first demonstration of the utility of chimeric antibody calibrators for homologous interpolation of specific human antibody levels. Chimeric antibody calibrators should be applicable to any diagnostic assay or kit designed to detect the presence of human antibodies specific for a given antigen (e.g., infectious agent, autoantigen, allergen, pharmaceutical).

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