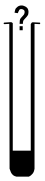
Total Human IgG EIA

An Enzyme Immunoassay for the determination of human IgG in oral fluid, serum and plasma samples

Cat. No: E14-105

For investigational use only  IVD

Manufactured & Distributed by

Clin-Tech Limited
Unit G Perram Works
Merrow Lane
GUILDFORD
GU4 7BN
UK

Phone: +44 (0)1483 302 007

Email: info@clin-tech.co.uk

INTENDED USE AND APPLICATION

For investigational use only. Not to be used for diagnostic purposes.

An enzyme immunoassay to measure levels of human IgG in body fluids such as oral fluid, serum, plasma and cerebrospinal fluid (csf) as a companion to the Microimmune Measles, Mumps and Rubella Capture EIA enzyme immunoassays.

This assay can be used to check that an oral fluid sample has been taken correctly and that there is sufficient specific IgG and IgM (if present) for a correct result in the Microimmune Measles, Mumps and Rubella Capture EIA enzyme immunoassays [(1)].

An IgG concentration of 2 mg/L is an indication that an oral fluid sample has been taken correctly and that the specimen is suitable for testing in both IgG and IgM Capture assays [(4)].

TEST PRINCIPLE

The assay is based on the double antibody sandwich ELISA format.

Polystyrene microplate wells are supplied pre-coated with anti-human IgG antibodies. Diluted test samples and IgG calibrators are incubated in the wells, during which IgG in the sample is captured. After a wash step to remove unbound material, the surface is probed for the bound IgG with an enzyme conjugated anti-IgG antibody. Following a second wash step, an enzyme substrate is added to the wells which produces a blue-coloured solution in the wells. The enzyme reaction is halted by the addition of Stop Solution (which also changes the solution yellow) and the absorbance is measured in a photometer. The IgG level in a test sample is related to the absorbance obtained and can be determined by the construction of a standard curve with the Calibrators provided in the kit.

WARNINGS AND PRECAUTIONS

- Wear disposable gloves when handling clinical specimens and kit components. Treat all test specimens and any materials that come into contact with them as potentially infectious.
- Although the serum used to prepare the Calibrators was not reactive for antibodies to HIV 1 and 2, HCV or Hepatitis B surface antigen, it should be handled and disposed of as though potentially infectious.
- TMB Substrate contains 3,3',5,5'-tetramethylbenzidine and has been reported to be non-carcinogenic. Contact with skin and mucous membranes should be avoided. Wear latex gloves when dispensing and using this reagent. If TMB Substrate comes into contact with skin and mucous membranes, rinse with copious amounts of water.
- Stop Solution contains 0.25 mol/L sulphuric acid. Contact with skin and mucous membranes should be avoided. If Stop Solution comes into contact with these sites, rinse with copious amounts of water.
- Good laboratory procedure should be employed to avoid cross contamination of samples and reagents. Take out only the required volume of reagent from the original container (usually 0.9 - 1.0 mL per strip) for dispensing into wells. Discard any unused reagent - do not return to the container!
- Avoid microbial contamination of reagents and do not use reagents that show signs of contamination.

- Do not mix components of one lot of kits with components from other lots.
- Dispose of clinical material and potentially infected materials in accordance with local regulations.

MATERIALS PROVIDED

The kit is stable up to the expiration date printed on the kit label if stored at 2-8°C.

	E14-105 (five plate kit) for up to 450 tests
ANTI-HUMAN IgG PLATE 8×12 microwell strips coated with anti-human IgG antibody packed in a re-sealable pouch with desiccant. Open the pouch by cutting along the notched edges and separating the re-sealable joint. Return unused strips to the pouch with desiccant and store at 2-8°C. Strips should be used within 3 months of initial opening.	PN 2109, 5 pouches
IgG CALIBRATORS: A set of pre-diluted six human IgG solutions, nominally containing 0, 0.025, 0.07, 0.2, 0.5, and 1.5 mg/L IgG (the exact value is printed on each bottle).	E14-022, 6×4 mL
TOTAL IgG TRACER. Affinity purified rabbit anti-IgG labelled with peroxidase.	E14-052, 60 mL
SERUM DILUENT: Phosphate buffered saline, protein stabiliser, detergent and red dye.	PN 20040, 2 × 500 mL
WASH BUFFER 10× Concentrated phosphate buffered saline, detergent and preservative. Dilute 1 in 10 with purified water before use.	PN 20024, 500 mL
TMB SUBSTRATE: A solution of 3,3',5,5'-tetramethyl-benzidine, a peroxide source and stabilisers.	R7-02, 60 mL
STOP SOLUTION: 0.25 mol/L sulphuric acid.	R5-02, 60 mL
PLATE INCUBATION BAGS: (self-seal polythene bags)	6

MATERIALS REQUIRED BUT NOT PROVIDED

- Oral Fluid collection device e.g. Oracol or similar device (see Specimen Collection).
- Buffer for extracting Oral fluid from an oral fluid collection device.
- Laboratory grade purified water, e.g. deionised or distilled water.
- Tubes suitable for diluting specimens and microtitre plate sealer (or bag).
- Micropipettes and disposable tips capable of delivering 1000 µL, 100 µL, 10 µL and 5 µL volumes.
- Waste discard container with disinfectant.
- ELISA plate reader capable of reading optical densities at 450 nm and 635 ± 15 nm.
- Incubator set to 37 ± 2°C.

SPECIMEN COLLECTION

Handle all oral fluid, blood, serum and plasma as potentially infectious material.

Oral fluid specimens should be collected as described on the outer package of the Oracol collection device. Other oral fluid collection devices should be validated in the assay before use.

Oral fluids should be eluted into transport medium, a buffer of neutral pH containing between 3-10% (v/v) foetal bovine serum, 0.2-0.5% (v/v) Tween-20 and antibacterial and antifungal reagents. The procedure for processing Oracol swabs used to collect oral fluid has been described in a video [(2)].

Serum and plasma (EDTA, citrated or heparinised) samples are suitable specimens for the test and should be obtained using standard procedure.

PROCEDURAL NOTES

The Calibrators are pre-diluted and contain human IgG at the level printed on the label. The Calibrators provided cover a standard curve over the range of 0 to 1.5 mg/L IgG. Add directly to wells when performing an assay: no dilution is required.

The accepted normal range for IgG in serum or plasma is 8 to 16 g/L. To measure IgG in this range, specimens can be diluted 1/40,000 in Serum Diluent. The IgG dose read off the standard curve must be multiplied by the full dilution factor used (40,000 in this example) to arrive at the correct IgG level in the specimen.

The amount of IgG in oral fluid specimen will depend on the device used and the extraction protocol. The expected level in a specimen taken using the Oracol device and extracted into 1 mL Transport Medium is 0.3 to 18 mg/L. To measure IgG in this range, specimens can be diluted 1/10 or 1/20 in Serum Diluent. The IgG dose read off the standard curve must be multiplied by the full dilution factor used (either 10 or 20 in these two examples) to arrive at the correct IgG level in the specimen.

REAGENT AND SAMPLE PREPARATION

Bring all reagents to room temperature (18-25°C) prior to use.

If necessary, warm the Wash Buffer 10× to re-dissolve any salts that may have formed on storage. Prepare working strength wash buffer by adding 1 part of the Wash Buffer concentrate to 9 parts distilled or deionised water. It is recommended that working strength buffer be prepared as required on the day of use. Any remaining working strength Wash Buffer should be stored at 2-8°C.

All other reagents are provided ready to use.

ENZYME IMMUNOASSAY PROCEDURE

1. Dilute all specimens in Serum Diluent as appropriate (see section above for guidance).
2. Remove and assemble the required number of microwell strips to perform the test. A minimum of 12 wells is needed for the Calibrators when run in duplicate and which must be included in each test run. Return unused microwell strips and the desiccant to the foil pouch and reseal.
3. Pipette 100 µL of each Calibrator to an assigned well and without delay pipette 100 µL of each specimen dilution to the assigned wells. Only test the number of samples in a single test run that can be dispensed within five minutes. Place microwell plate in a sealed plastic bag or cover with sealing tape. *Note: this step can be accomplished more quickly if controls and test samples are pre-dispensed into 1.1 mL microplate-compatible tubes or a microtitre holding plate then transferred to the test plate using a multichannel pipette*.

   Incubate at 37°C for 30 ± 2 minutes.
4. Wash wells four times with working strength Wash Buffer (see reagent preparation). The wash cycle is carried out as follows: aspirate the contents of the well and dispense 350 µL/well of diluted wash buffer, leave to soak for approximately 30 seconds and aspirate. Repeat the wash cycle three further times. It is recommended to use an automatic plate washer for this procedure. Tap the wells dry on absorbent paper.
5. Pipette 100 µL of Total IgG Tracer to each well, cover plate and incubate at 37°C for 30 ± 2 minutes.
6. Wash the wells four times with working strength Wash Buffer as in step 3.
7. Pipette 100 µL of TMB Substrate to each well. This is best performed with a multichannel pipette. Incubate for 10 ± 1 minutes, protected from strong light at room temperature (18-25°C).
8. Pipette 100 µL of Stop Solution to each well. This reagent should be added to wells in the same order as the previous step so that the timing is accurate.
9. Read the optical densities (OD) at 450 nm in an ELISA plate reader. If the feature is available, set the reference wavelength between 620 and 650 nm.
10. Construct a standard curve using the optical density obtained for each Calibrator. It is recommended to fit the curve using four parameter logistic curve-fitting software.
11. Verify the assay (see below).

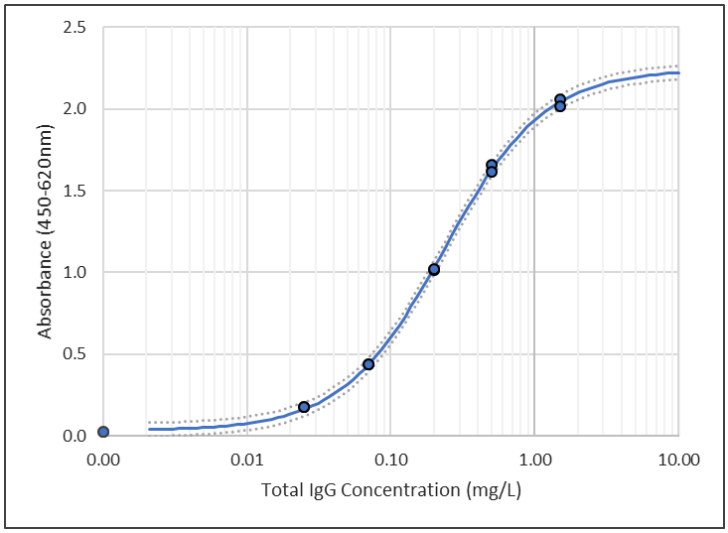
12. Read the dose obtained for each test specimen and correct for the dilution used to obtain the dilution-corrected IgG level.

QUALITY CONTROL.

The optical density $OD_{450\text{-}620\ nm}$ of the Top Calibrator should be greater than 1.5.

The $OD_{450\text{-}620\ nm}$ of each of the Zero Calibrator should be less than 0.15.

EXPECTED RESULTS



Representative standard curve

Optical density readings obtained for each calibrator were fitted to a four-parameter logistic curve routine in Microsoft Excel[3]. The line of best fit and 95% confidence intervals are shown. This graph is representative only and should not be used in place of data generated at the time of each assay.

Plasma samples obtained from normal healthy blood donors were tested in the Total IgG EIA. The IgG was 11.2 ± 6.0 g/L (mean ± 2 SD).

LIMITATIONS OF THE TEST

Microbiological contamination of the specimens may lead to erroneous results.

The suitability of other oral fluid collection devices and oral fluid extraction buffers has not been established.

REFERENCES

1. Perry KR, Brown DWG, Parry JV, Panday S, Pipkin C and Richards A (1993). Detection of measles, mumps and rubella antibodies in saliva using antibody capture radioimmunoassay. J. Med. Virol. 40, 235-240.
2. Medical training video: oral fluid samples. Public Health England (2013). Accessed 04-Dec-2015 at https://www.youtube.com/watch?v=6wDDLp_OaTc.
3. Brown A (2001). A simple step-by-step guide to non-linear regression analysis of experimental data using a Microsoft Excel spreadsheet. Computer Methods in Biomedicine 65, 191-200.
4. Brown K. Personal communication.

WARRANTY

The product is warranted to perform as described in the labelling and in the pack insert when used as instructed. NO WARRANTY EXTENDS BEYOND THIS. CLIN-TECH LTD DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. The seller's sole obligation and the purchaser's exclusive remedy for breach of this warranty shall be at the option of Clin-Tech Ltd to replace the products. In no event shall Clin-Tech be liable for any proximate, incidental or consequential damage in connection with this product.

SUMMARY OF ASSAY PROTOCOL

Bring All Reagents to Room Temperature

Dilute Wash Buffer 10× in water (1 + 9) as required.

Prepare diluted test specimens as appropriate for the sample type

	Volume per well	**Incubation Time and Temperatures**
1. Assemble required number of coated strips into plate frame		
2. Pipette Calibrators and diluted test specimens. Complete this step within 5 minutes.	100 µL	30 ± 2 min at 37°C
3. Wash with diluted Wash Buffer	4 × 350 µL	
4. Pipette Total IgG Tracer	100 µL	30 ± 2 min at 37°C
5. Wash with diluted Wash Buffer	4 × 350 µL	
6. Pipette TMB Substrate	100 µL	10 ± 1 min protected from light at room temperature
7. Pipette Stop Solution	100 µL	
8. Read Optical Density at 450 nm with reference set to 635 ± 15 nm		
9. Construct a standard curve, read values of this curve and correct unknown sample concentrations by the dilution to arrive at the final sample concentration.		

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