FAQ: Frequently Asked Questions

Device: Allerquant™ Foods IgG ELISA (90G, 45G, 21, 14G, 7G, 4G, & Custom)

Sample Type

What type of samples can I collect and test?
The determination of food-specific IgG antibody should be performed with serum. Collect whole blood aseptically into an SST™ tube according to approved venipuncture techniques, fasting is not required. Mix blood well by gently inverting blood SST™ tubes 5 times. Place the SST™ tube in a rack in an upright position and allow the blood to clot at room temperature for 30 minutes. After allowing the clot to form, immediately (but no later than 2 hours), centrifuge the specimen(s) at room temperature at a speed equivalent to 1000g – 1300g RCF for 10 minutes in a swing bucket centrifuge or for 15 minutes in a fixed angle centrifuge. Transfer the serum into an appropriate tube and store at 2-8°C for short term storage prior to testing.

What should I do if the test sample is hemolyzed or lipemic?
Grossly hemolyzed, lipemic, and icteric samples as well as samples containing particulate matter or showing obvious microbial contamination should not be tested.

Is it possible to tests with plasma?
Our Allerquant ELISA kits have only been validated on Human Serum samples. We do not have any data to support use with Plasma samples.

How long can samples be stored before testing?
Serum samples may be stored up to 14 days at 2-8°C. Serum samples frozen at -70°C are stable for up to 3 months with one freeze thaw cycle.

Can I test non-Human samples?
At this time, Biomerica has not validated its Allerquant™ Foods IgG ELISA kit with the use of non-Human serum.

Reagents

Can I interchange lot numbers?
ELISA kit components are lot specific. Do not interchange lot numbers from previous kits.

What are the storage conditions for the kit components?
Store all reagents at 2-8°C. All reagents except the Calibrators and the Wash Concentrate are ready-to-use.

How quickly should I pipette calibrators, controls and samples into the wells?
For each of the prepared working Calibrator dilutions to calibration curve, Calibrator 50 U/ml through 400 U/ml, add Calibrators to wells immediately after serial dilution is complete to designated wells according to IFU.

What is the stability of the Calibrators after preparation?
Use the calibrators and controls as soon as possible after preparation of calibration curve. Discard working Calibrator dilutions to calibration curve after each use.

What is the stability of the working wash solution?
The diluted working wash solution is stable for 6 months at 2-8°C.

Is crystal precipitate present in the wash concentrate normal?
Crystal precipitate present in the Wash Concentrate is normal. This is due to storage at lower temperature such as 4°C. Dissolve by placing the vial in a 37° C water bath or incubator for 30 minutes.

When should I mix Substrate Solution "A," and "B," in equal proportions?
Substrate Solution "A," and "B," should be mixed in equal proportions 30 minutes prior to use. Mixed substrate solution is stable for 60 minutes at room temperature and should be kept away from light until use.

ELISA Testing Procedure

How long does it take to perform the Allerquant™ Foods IgG ELISA?
Assay time is 1 hour 40 minutes.

What types of antibodies are used in the Allerquant™ Foods IgG ELISA kit?
Enzyme labeled antibody conjugate is allowed to react with allergen-antibody complex.
The enzyme labeled antibody conjugate is a Food IgG labeled with horseradish peroxidase [HRP] for detection.

What types of IgG are detected in the Allerquant™ Foods IgG ELISA kit?
Our food IgG ELISA detects the total IgG, which is a combination of all IgG subclasses IgG1, IgG2, IgG3, IgG4. This allows for an increase in the diagnostic sensitivity of the test when compared to testing for only IgG4.

Is there interference with human IgE, IgA and IgM?
Interference from human IgE, IgA, and IgM is negligible.

What Precaution should I take when running this kit?
The source of the Calibrators and Controls is human serum, in which the human serum used has been found non-reactive to HbsAg, anti-HIV ½, and anti-HCV when tested with FDA licensed reagents. Although there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent; these reagents should be handled as if potentially infectious.

Some reagents contain sodium azide as a preservative. Sodium azide may react with lead, copper, or brass to form explosive metal azides. When disposing of these materials, always flush with large volumes of water to prevent azide buildup.

Stopping Solution consists of 1 N Sulfuric Acid. This is a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves and eye protection, with appropriate protective clothing. Any spill should be wiped immediately with copious quantities of water. Do not breathe vapor and avoid inhalation.

Substrate Solution A contains Dimethyl Sulfoxide (DMSO). DMSO is a skin irritant and can also cause irritation to the mucosal membranes and upper respiratory tract if inhaled, or ingested. Avoid exposure by wearing personal protective equipment such as gloves and safety glasses. If skin or eye contact occurs, flush with water for a minimum of 15 minutes. If inhaled, move to fresh air. If ingested, obtain medical attention.

A sulphurous odor may be present in the Substrate Solution A and will not affect ELISA results. The handling of Substrate Solution A and preparation of the Working Substrate Solution should be done in a fume hood or a well-ventilated area to minimize exposure.

All specimens, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. Common precautions in handling should be exercised, as applied to any untested patient sample.

Can I use an automated microplate washer when running the ELISA?
Yes, an automated plate washer may be used. If a microplate washer is unavailable, manual washing is also acceptable.

Can I run my assay via automated methods?
Biomerica has only validated its Allerquant™ Foods IgG ELISA kit via manual testing as outlined in the approved IFU. You would need to perform your own validation to ensure equivalent performance between the manual method and the chosen automated method.

What wavelength does the assay need to be read at?
Set the microplate reader at 450 nm and read the absorbance in all the wells.

Interpretation of Results

What concentration units are expressed in the test result?
The Biomerica Allerquant™ Foods IgG ELISA kit concentration unit results are in U/ml.

Curve Fit, QC, and Data Analysis

What type of curve fitting method is acceptable to interpret data results?
Computer programs using cubic spline, point to point, or Quadratic can interpolate the patient values from the calibration curve.

What is considered an invalid Test?
For the test to pass, it must meet the following Q.C. specifications for O.D. (Optical Density) at 450 nm.

O.D. Well 1A	< 0.2
O.D. Well 1B	> 1.2 x OD 1A
O.D. Well 1C	> 1.2 x OD 1B
O.D. Well 1D	> 1.2 x OD 1C
O.D. Well 1E	> 1.2 x OD 1D
Concentration Positive	> 100 U/ml

What is recommended for internal QC of each kit run?
It is recommended that control serum or serum pools should be evaluated with each run of calibrators and patient samples. Results generated from the analysis of the control samples should be evaluated for acceptability using appropriate statistical methods.

What should I do if the sample result is higher than the highest Calibrator?
Samples with concentration values higher than the highest calibrator >200 U/ml are interpreted as being highly intolerant.

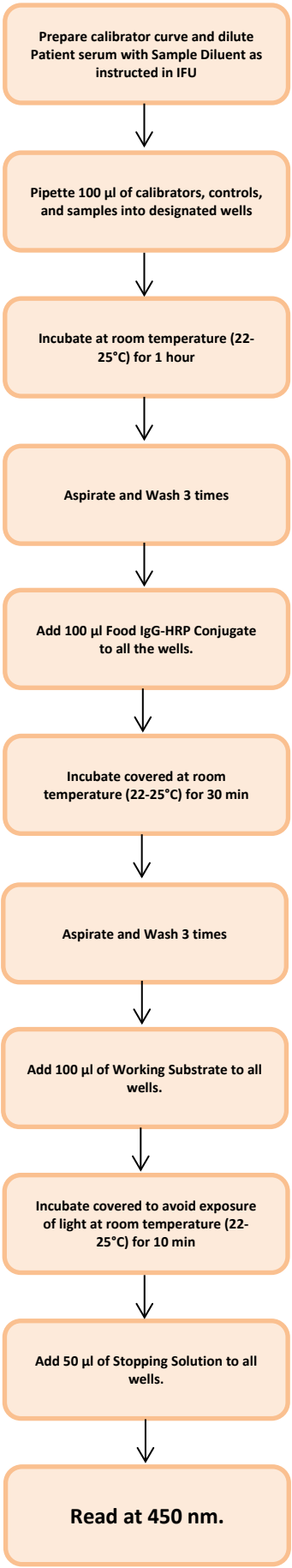
The absorbance readings, after extrapolation as Biomerica U/ml, should be interpreted as follows for each allergen or extract.

READING	INTERPRETATION	
< 50 U/ml	Negative	**0**
50 - 100 U/ml	Mildly Intolerant	**+1**
100 - 200 U/ml	Moderately Intolerant	**+2**
> 200 U/ml	Highly Intolerant	**+3**

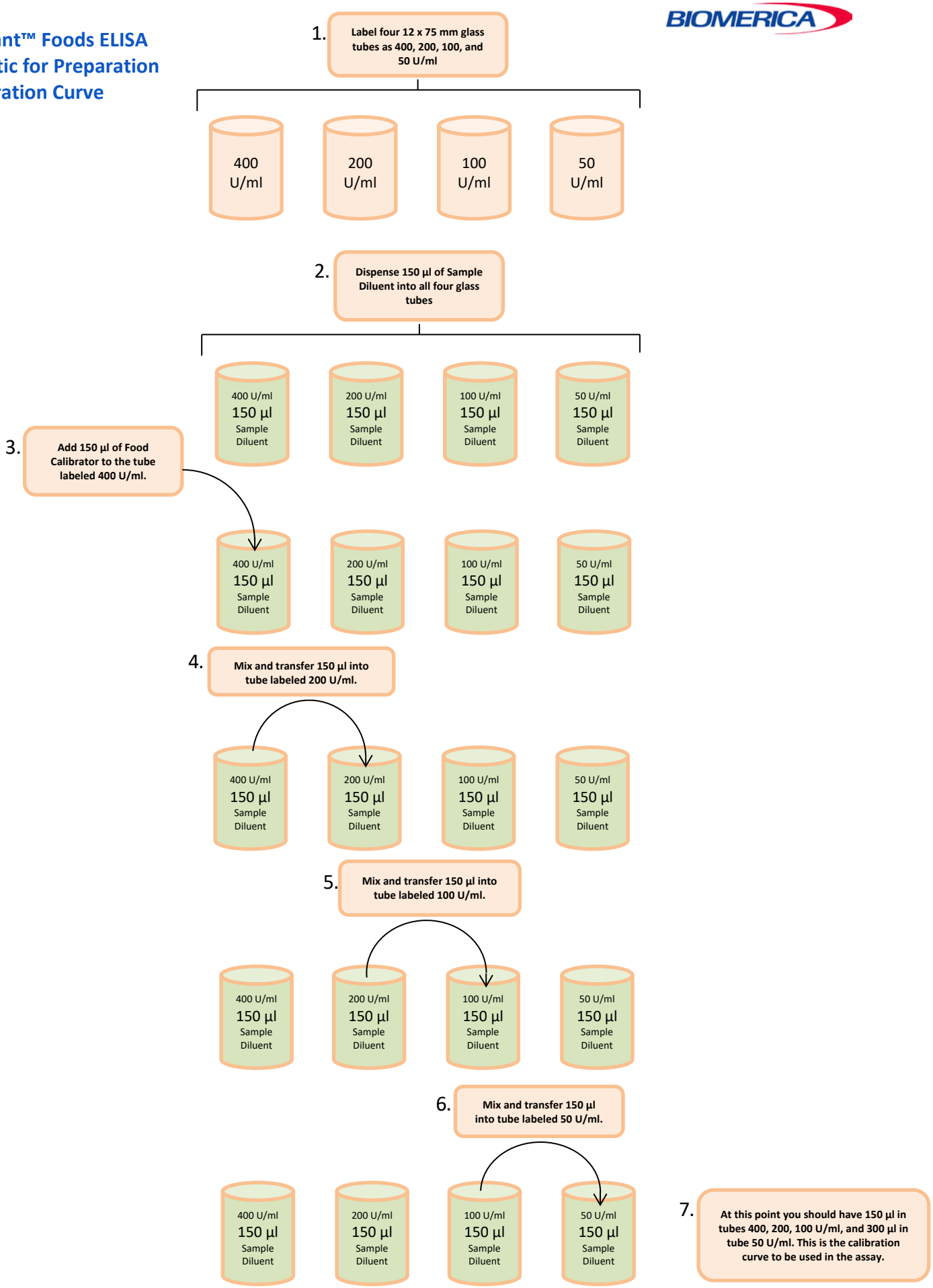
Troubleshooting Tips

Observation	Probable Cause
Low Absorbance	Improper calibrator/control preparation
	Enzyme or substrate reagent degradation
	Improper buffer/diluent used
	Reagents were cold when added to wells
	Incorrect incubation time
	Wells dried out
	Excessive wash/aspiration pressure from automated plate-washer
	Plate reader set at incorrect wavelength
High Background	Incomplete washing/aspiration of wells
	Substrate contamination
Poor Precision	Inaccurate pipetting
	Inadequate mixing of reagents
	Samples contain precipitates
	Wells dried out
	Contaminated well bottom
Poor Standard Curve	Improper dilution/handling of calibrators/controls
	Incomplete washing/aspiration of wells
	Inaccurate pipetting
	Improper diluent used for calibrator/control preparation

Allerquant™ Foods ELISA Procedural Flow Chart



Allerquant™ Foods ELISA Schematic for Preparation of Calibration Curve



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