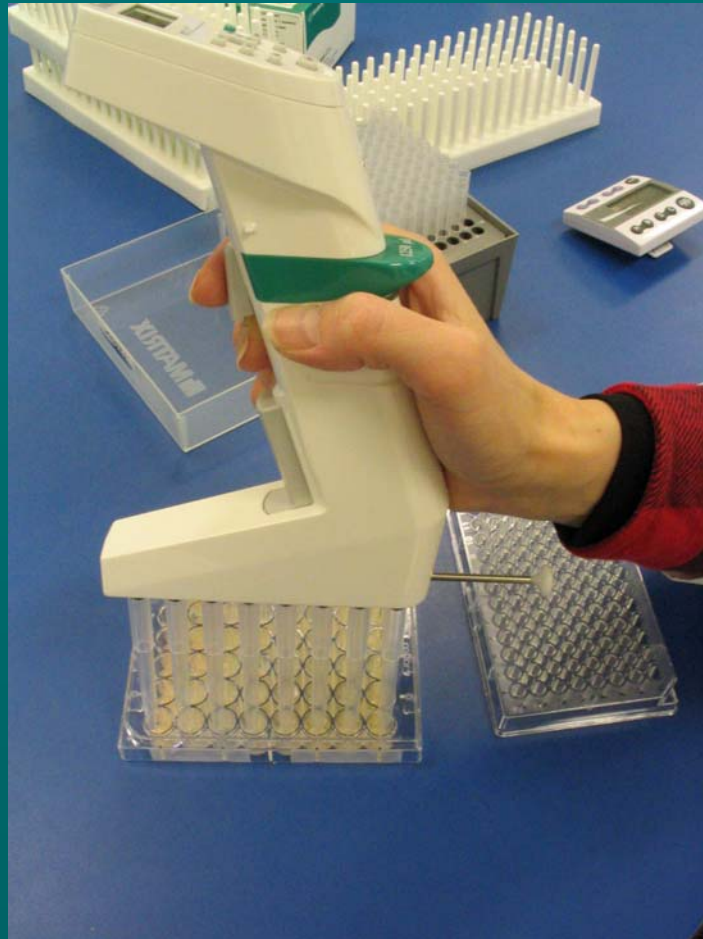


Proper Pipette Technique

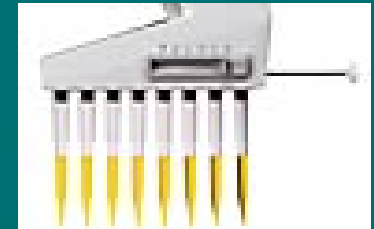
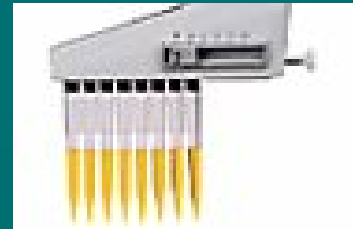
Jamie Welch, EnviroLogix Inc.



Proper Pipette Technique

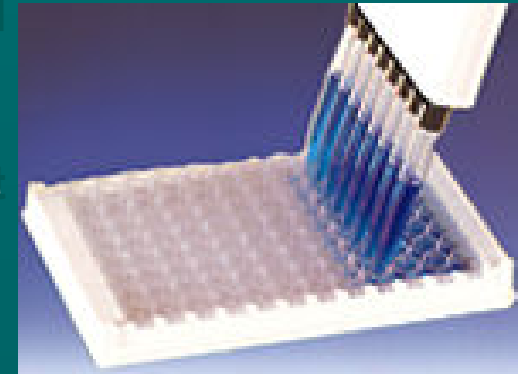
■ Types of pipettes:

- Fixed Volume Single Channel
- Single Channel Adjustable
- Multichannel Adjustable
- Digital Multichannel
- Digital Multichannel Expandable



■ When to use multichannel and single-channel pipettes:

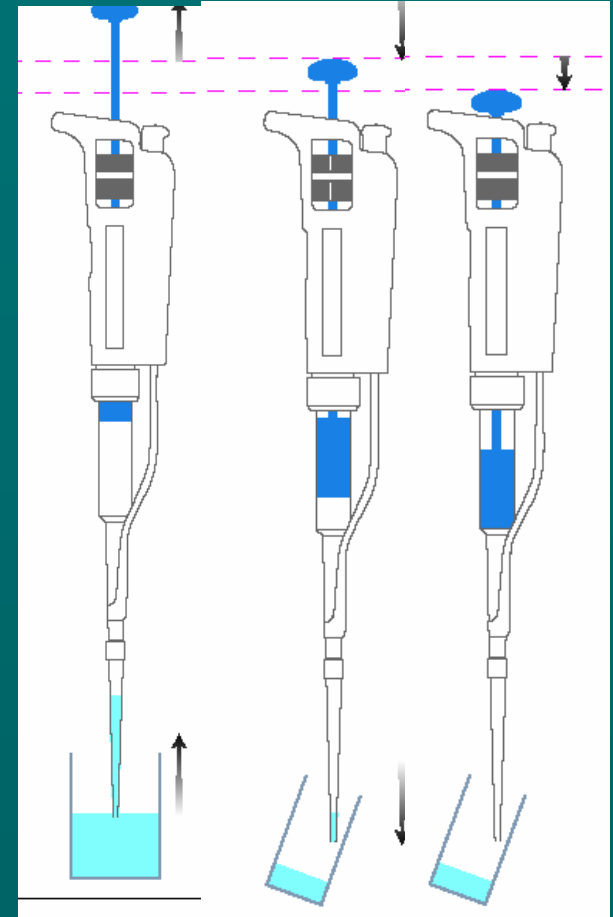
- When ELISA's require greater than 10 minutes to pipette samples into wells it is recommended to use a multichannel pipette.
- Delta Time Effect: The difference in incubation time between the first well pipetted and the last well which leads to longer incubation times for the first wells.
- Some assays may be more sensitive to this delta time effect because of the antibody's affinity for the analyte.



Reverse vs. Forward Pipetting

Forward Pipetting:

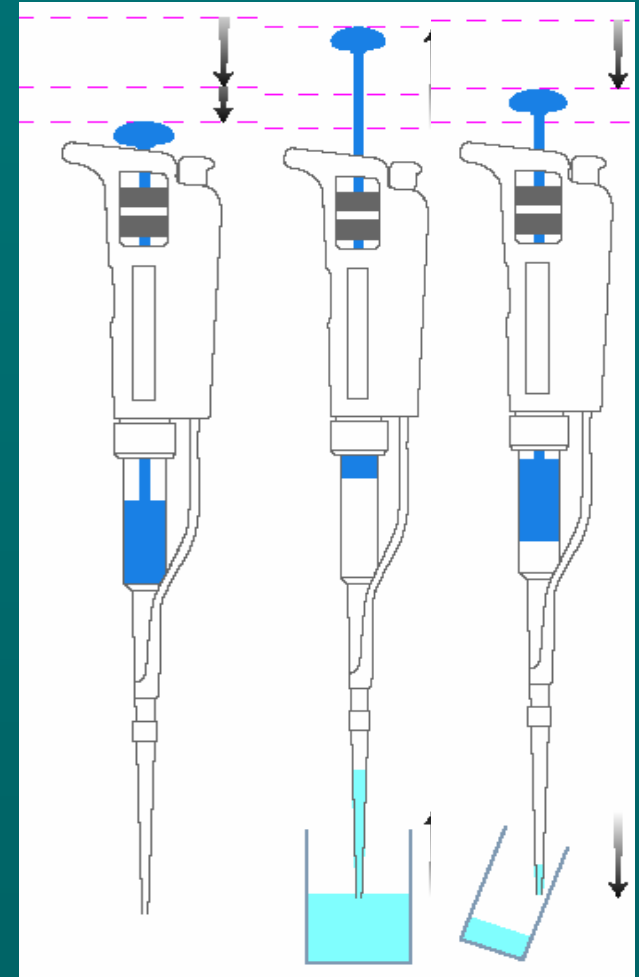
- For Standard solutions including buffers, water, dilute saline, and dilute acids and bases.
- More accurate and precise results than reverse pipetting.
- Entire volume of liquid aspirated into the pipette tip is dispensed.
- Depress the push button to the first stop.
- Place the tip 1 cm below the surface of the liquid and draw solution slowly up. Withdraw tip touching it against the sides of the reservoir to remove excess liquid.
- Deliver liquid sample by depressing push button to the first stop, and then blowing out the remaining liquid by depressing to second stop.



Reverse vs. Forward Pipetting

Reverse Pipetting:

- For high viscosity solutions, small volumes, buffers with detergent, and solutions that foam easily.
- Depress push button all the way to the second stop.
- Place the tip 1 cm below the surface of the liquid and draw solution slowly up. Withdraw tip touching it against the sides of the reservoir to remove excess liquid.
- Deliver solution by depressing the push button to the first stop. A portion of the solution will remain in the pipette tip. This volume should not be delivered.



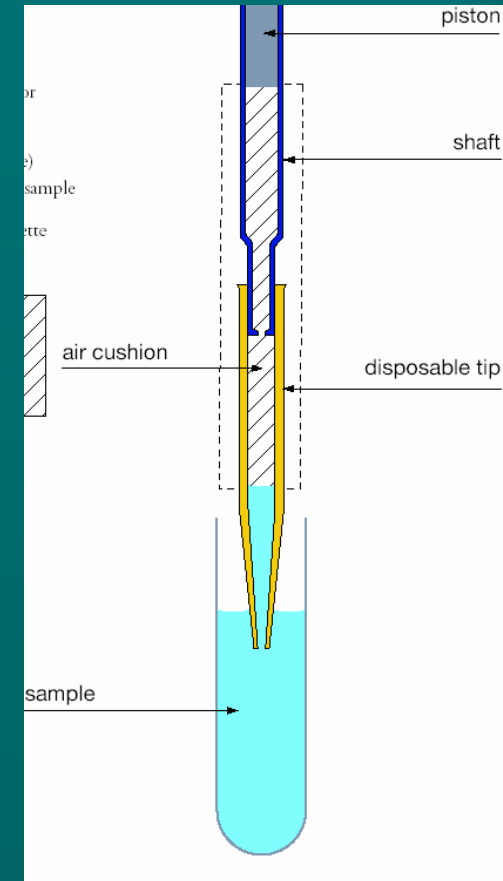
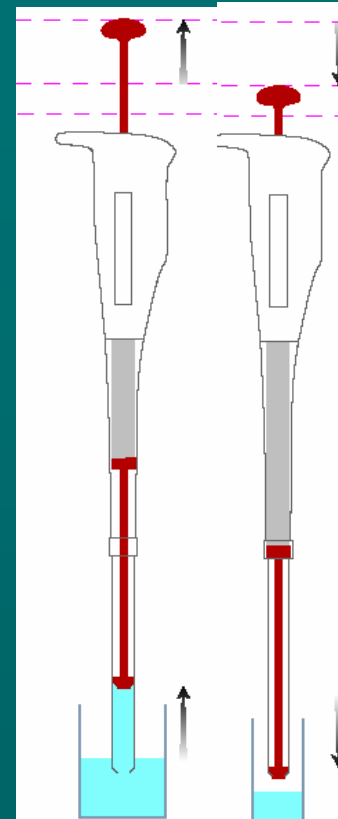
Positive Displacement vs. Air Displacement Pipettes

Air Displacement:

- Precise draining by air pressure (air cushion)
- For normal solutions and buffers

Positive Displacement Pipettes:

- For viscous, dense, volatile, radioactive or corrosive samples
- Direct contact of samples with piston
- Contains an internal mechanism for wiping off pipette barrel



Positive Displacement vs. Air Displacement Pipettes



General Pipette Technique

Manufacturer Recommendations

In order of importance:

- 1.** Wet pipette tip 2-3 times with working solution before initiating work. Pre-wetting increase the humidity within the tip and decreases sample evaporation. Using the same tip without pre-wetting to deliver multiple samples will result in a lower volume for the first few samples.
- 2.** Make sure pipettes, tips, and solutions are at the same temperature. The volume of sample delivered by air displacement pipettes varies with air pressure, relative humidity, and vapor pressure. Working with all components at a constant temperature minimizes variation.

General Pipette Technique

Manufacturer Recommendations

- 3.** Examine pipette tip before dispensing sample. Make certain that no air bubbles are present in the sample, and that the full sample volume was aspirated. Only clean the pipette tip when liquid is visible on the outside of the pipette. If present clean carefully and do not touch tip opening with absorbent material. Unnecessary wiping could lead to sample loss.
- 4.** Keep tip in sample for one or two seconds after aspiration. The amount of liquid in the tip “bounces” slightly when the plunger stops. Slow, even plunger release and a pause after aspiration will minimize errors resulting from this phenomenon.

General Pipette Technique

Manufacturer Recommendations

- 5.** Hold pipette in an upright position when aspirating solution; within 20 degrees of vertical. This is most important with samples below 50 μ L. Surface tension causes the samples to vary if the exit angle varies. Touching the tip against the side of the well will result in a loss of sample.
- 6.** Place pipette down between sample deliveries. The heat from a hand will be transferred to the pipette and will disrupt the temperature equilibrium and can effect sample volume.

General Pipette Technique

Manufacturer Recommendations

7. Immerse pipette tip into the solution to the proper depth; 2 to 5 mm below the meniscus and away from the walls and bottom during sample aspiration. Inserting the tip too deep into the sample causes droplets to form on the outside of the tip. If the pipette is not immersed deeply enough, sample vortexing will occur leading to inaccurate aspiration.

One pipette manufacturer recommends the following immersion depths:

volume μl	immersion depth mm
0.1 - 1	1
1 - 100	2-3
101 - 1000	2-4
1001 μl - 10 ml	3-6

General Pipette Technique

Manufacturer Recommendations

- 8.** Use correct fitting pipette tips and attach tips firmly so that a good seal is established. Quality tips have a thin flexible plastic wall that allows for an airtight seal and dependable sample delivery.
- 9.** Use a constant plunger speed and pressure, especially with viscous solutions. A rapid aspiration speed can lead to the sample entering the pipette chamber. Fluid in this chamber can result in pipette inaccuracy and contamination.

General Pipette Technique

Manufacturer Recommendations

- When setting the pipette volume always turn the plunger counterclockwise until the volume indicator is 1/3 of a revolution above the desired setting, then slowly turn the plunger clockwise to the desired setting. Always dial down to the desired number. This prevents mechanical backlash from affecting accuracy. The friction prevents unintentional volume changes.
- Never lay a pipette with a sample on its side. Chamber contamination can result.
- Most importantly, maintain a consistent procedure so that calibrators and sample are treated uniformly.

Pipette Calibration and Cleaning

Jamie Welch, EnviroLogix Inc.



Pipette Calibration

Frequency of Calibration

- It is recommended to calibrated pipettes every 3 months.

Single Channel Pipette Calibration Procedure

- Use an analytical calibrated balance with an accuracy of 0.0001g.
- Use non-aerated distilled or deionized water.
- Perform calibration in a draft free room or with an enclosed balance.
- Maintain the relative humidity above 55% to decrease evaporation effects. Air humidity should be as high as possible especially for volumes below 50 μ L.



Pipette Calibration

Single Channel Pipette Calibration Procedure:

- Pipette, water, and air should be the same temperature, between 15-30C.
- Test maximum pipette volume and minimum volume or 10% of maximum volume (which ever is greater).
- Pre-wet tips.
- Test 10 volumes per setting.

Multi-channel Pipette Calibration Procedure:

- Use single channel procedure for all 8 or 12 channels.

Pipette Calibration

Calibration Acceptance Criteria

- Accuracy (mean error): How close a measurement is to the accepted value. Difference between the dispensed volume and the selected volume of a pipette.
- Precision (random error): Indicates how close together or how repeatable the pipette volumes are. A precise measuring instrument will give very nearly the same result each time it is used. Precision is expressed as standard deviation (S) or coefficient of variability (CV).



Accuracy



Precision



Accuracy and Precision

Pipette Calibration

Calibration Acceptance Criteria Calculations:

MEAN WEIGHT: Result expressed in mg.

$$\bar{w} = \frac{\sum_{i=1}^n w_i}{n}$$

\bar{w} = mean weight

n = number of measurements

w_i = individual weighings

MEAN VOLUME: The mean weight result with corrections for evaporation and Z-factor.
Expressed in μL .

$$\bar{v} = (\bar{w} + \bar{e}) \times Z$$

\bar{w} = mean weight (mg)

\bar{v} = mean volume

\bar{e} = evaporation rate (mg)

Z = Z-factor

Pipette Calibration

Calibration Acceptance Criteria Calculations:

MEAN ERROR: The difference between the mean volume of actual measurements and the true value as specified by the volume setting of the pipette. Expressed in μL .

$$E = \bar{v} - v_0$$

E = mean error
 \bar{v} = mean volume
 v_0 = volume setting

As a percentage

$$E \% = \frac{\bar{v} - v_0}{v_0} \times 100$$

STANDARD DEVIATION: Quantifies the magnitude of scatter due to random error.

$$s = \sqrt{\frac{\sum_{i=1}^n (\bar{w} - w_i)^2}{n-1}}$$

s = standard deviation
 n = number of weighings
 \bar{w} = mean weighing
 w_i = individual weighings

As a percentage, also known as coefficient of variation (CV)

$$S \% = \frac{S}{\bar{v}} \times 100$$

Pipette Calibration

Calibration Acceptance Criteria Calculations:

MODEL VOLUME	VOLUME SET μ L	ACCURACY		PRECISION	
		%	μ L (\pm)	%	μ L (\leq)
P-2	0.2	12.0	0.024	6.0	0.012
	1	2.7	0.027	1.3	0.013
	2	1.5	0.030	0.7	0.014
P-10	1	2.5	0.025	1.2	0.012
	5	1.5	0.075	0.6	0.03
	10	1.0	0.1	0.4	0.04
P-20	2	7.5	0.15	2.0	0.04
	10	1.5	0.15	0.5	0.05
	20	1.0	0.2	0.3	0.06
P-100	10	3.5	0.35	1.0	0.1
	50	0.8	0.4	0.24	0.12
	100	0.8	0.8	0.15	0.15
P-200	50	1.0	0.5	0.4	0.2
	100	0.8	0.8	0.25	0.25
	200	0.8	1.6	0.15	0.3
P-200-M8	20	2.5	0.5	1.25	0.25
	100	1.0	1.0	0.5	0.5
	200	1.0	2.0	0.5	1.0
P-1000	100	3.0	3.0	0.6	0.6
	500	0.8	4.0	0.2	1.0
	1000	0.8	8.0	0.15	1.5
P-5000	500	2.4	12.0	0.6	3.0
	2500	0.6	15.0	0.2	5.0
	5000	0.6	30.0	0.16	8.0
P-10ML	1 mL	5.0	50.0	0.6	6.0
	5 mL	1.0	50.0	0.2	10.0
	10 mL	0.8	80.0	0.16	16.0

Pipette Calibration

Pipette Calibration Excel File:

Pipette #		Temp. °C	21
Volume mL	0.2	$\rho=$	0.998022
	Reading		
R1	0.2001		
R2	0.1996		
R3	0.1995		
R4	0.2005		
R5	0.2006		
R6	0.1999		
R7	0.2012		
R8	0.1997		
R9	0.2012		
R10	0.2007		
Pass/ Fail	PASS	$x=$	0.2003
$x/\rho=$	0.2007	$x/\rho-V=$	0.0007
Precision (%CV)	0.3158	% Accuracy	0.35

Pipette Cleaning

- Pipette cleaning should be performed according to manufacturer recommended procedures.
- Gross cleaning can be accomplished and can improve the performance of the pipette. It is recommended that the P-2 and P-10 pipettes are cleaned by professionals, as they include miniaturized parts.
- Cleaning is commonly accomplished with distilled water or isopropyl alcohol and a lint free tissue.
- After cleaning pipettes should be re-calibrated.
- Some pipettes include adjustment tools so that pipettes can be brought into calibration.
- If cleaning and adjustment do not improve pipette calibration performance, pipettes should be sent to a certified facility for cleaning and calibration.



Non-Gravimetric Pipette Calibration

Alternatives to gravimetric pipette calibration:

- Artel: Colorimetric means for calibrating pipettes.



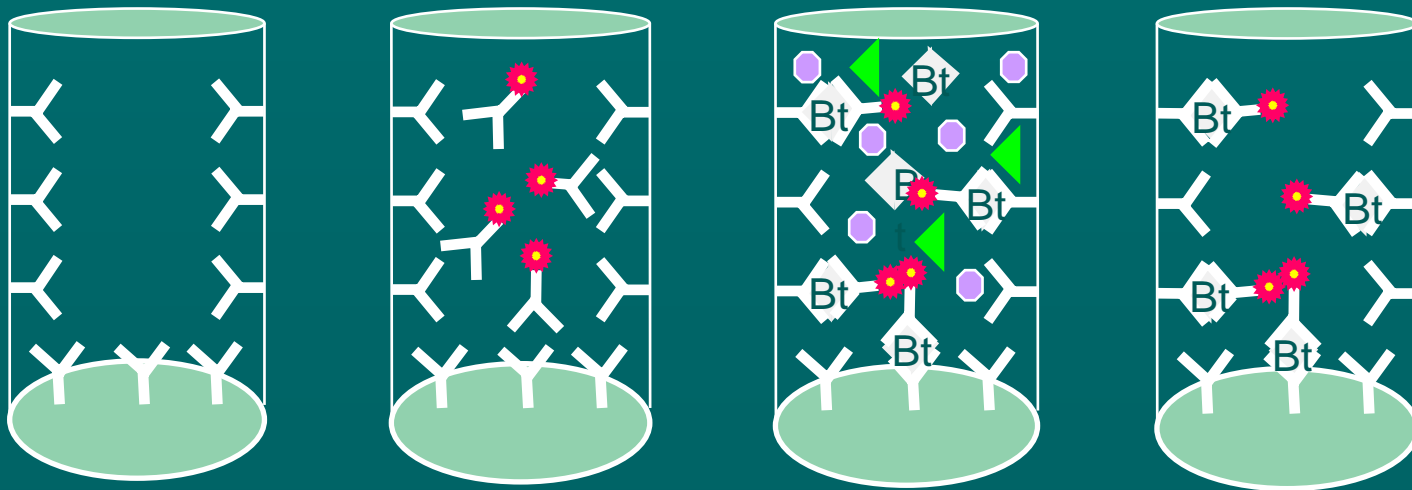
Hook Effect in ELISA

Jamie Welch, EnviroLogix Inc.



Hook Effect in ELISA

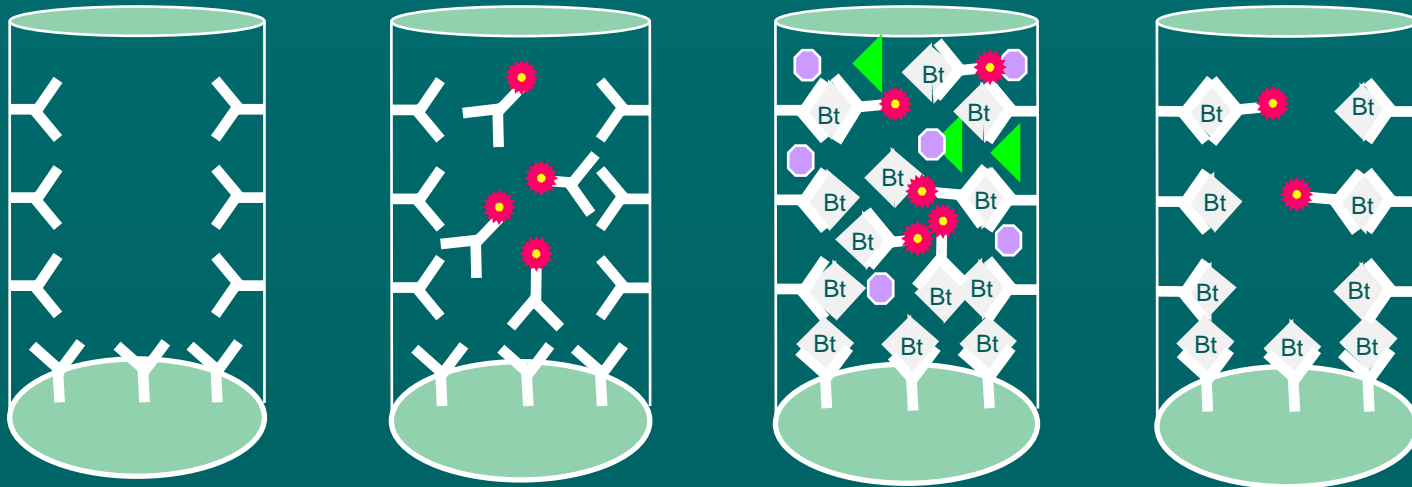
- **Hook Effect:** When an ELISA system is overwhelmed with the target antigen resulting in lower than expected OD readings for lower dilution samples than higher diluted samples.
- **Normal Sandwich Assay:** Antigen is sandwiched between the antibody coated on the plate and the conjugate.



Hook Effect in ELISA

- The antibody coated on the plate and conjugate antibody have their antigen binding sites occupied so that the antigen can not be sandwiched between the two antibodies.

Hook Effect Sandwich Assay:



Hook Effect in ELISA

What events or sample types are likely to cause a hook effect?

- Pure GMO protein from life sciences companies.
- RR soy leaf (sprouts)
- RR soy bulk grain can cause a hook effect in assays (200-250ppm)

Usually only a problem when quantifying protein level

- High protein concentrations will still produce positive ELISA results, however, the ELISA OD's will be suppressed.

Hook Effect in ELISA

When to consider hook effect in plate assays:

- When a neat or undiluted sample has a lower OD than further sample dilutions:

Ex:	<u>Dilution</u>	<u>OD</u>
	Neat	3.42
	1:2	3.87
	1:4	3.93
	1:8	3.87
	1:16	3.74
	1:32	3.46
	1:64	2.69
	1:128	1.94

Hook Effect in ELISA

Techniques to prevent Hook Effects:

- Run dilutions of samples to look for unexpected results.
- Know your sample source and the expected protein concentration to include the appropriate dilutions in your assay.
- Tests manufacturers:
 - Use a higher antibody concentration to coat the plate or a higher conjugate concentration.
 - Select antibodies that have a weaker attraction for the target protein. (antibody binding sites will not be occupied as quickly).

Two types of hook effects:

- Antigen excess
- Over coating of plates with monoclonal antibody