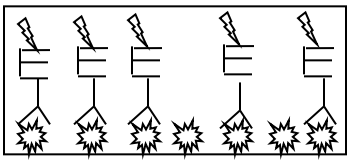
Example EIA Assay SOP:
Enzygnost Measles & Rubella IgG ELISA procedure using Dried Blood Spot (DBS) samples

This enzyme-linked immunosorbent assay (ELISA) detects IgG antibodies elicited in response to measles and rubella virus infections or vaccination in human blood. The sample wells in the odd numbered columns are coated with dried measles or rubella virus-infected Vero cells to which IgG antibodies bind. Even numbered columns are coated with dried uninfected Vero cells, which serve as a negative control that quantifies any non-specific binding by any antibody in the patient's serum. The anti-human IgG conjugate binds to IgG in the patient's serum and is conjugated (attached) to an enzyme that reacts with the substrate chromogen solution. Because the conjugate will bind any human IgG antibody (not just measles or rubella antibodies) and the substrate chromogen solution will react whether the conjugate enzyme is bound or unbound, washing with the wash solution after adding sera and conjugate is critical to remove any unbound IgG antibodies or conjugated enzyme. Therefore, a proper reaction looks like:



Name in kit/Traditional Name
Chromogen Solution/SUBSTRATE
α-human IgG Conjugate/CONJUGATE
Patient's IgG /ANTIBODY
MV-infected Vero cells/ANTIGEN

PREPARATION

1) Remove reagents from refrigerator to bring to room temperature.

2) Determine the samples you will run and the number of plate columns you will need (Remember, each sample is added to both measles antigen well (odd-numbered columns) and control well (even-numbered columns).

3) Check reagent volumes to ensure that you have all necessary components. Obtain appropriate materials: 20uL pipette, 1000uL pipette, multichannel pipette, reagent reservoirs, hole puncher, scrap paper, microcentrifuge tubes, forceps, PBS+Tween+milk, 15mL conical tube.

4) Make reagents that will be needed throughout the procedure:
   a.) α-human IgG/ Conjugate = 250uL Conjugate (green) + 12.5mL Conjugate Buffer (Microbiol G)
   b.) BLUE sample buffer = 2.5mL Color solution (blue) + 50mL Sample Buffer POD (Diluent)
   c.) Washing Solution = 20mL Washing Solution + 400mL distilled water
   d,) Milk elution buffer = 2.5g powdered milk+250uL Tween 20 + 50mL PBS

ELUTE DBS

1) **Label 1 microcentrifuge tube** for each patent sample for eluting the DBS.
   a. This will be enough to run both the measles and rubella assays

2) Punch out 2 spots from patient card, fold in half, and place in labelled microcentrifuge tube. Repeat for all samples.
   a. Note: 2 spots Is one for each of the assays as this is for both

4) Add **250uL milk elution buffer** to each microcentrifuge tube.

5) Pulse vortex 5 times. Incubate for **1 hour at room temperature** and vortex every 15 minutes.

6) Centrifuge microcentrifuge tubes containing DBS and elution buffer for **30 minutes at 1800x*g***.

7) Label 96 well plates according to the plate maps for alliquoting and storing eluate

8) Alliquote 50uL of eluate into labeled wells of the 96 well plates – Note: there should be one plate for measles and one for rubella. This is done to cut down the time needed to extract the eluate from the centrifuge tubes to maximize volumes.

9) Prepare capillary blood – for each sample label a microcentrifuge tube.
a) Add 400uL BLUE sample buffer to each tube.
b) Add 20uL of sample to the BLUE sample buffer in each tube
c) Vortex

9) Add **400uL BLUE sample buffer** to an additional microcentrifuge tube. Add **20uL Reference P/N** to microcentrifuge tube containing 400uL BLUE sample buffer. This will serve as your positive control. DO NOT DILUTE DBS SAMPLES.

ELISA

1) Label the top of the columns (*i.e.*, 1, 3, 5, 7, 9, 11) in case they dislodge during washing and **add 170uL Uncolored sample buffer (Diluent)** to each well for a **DBS sample. (columns 1-6).**

2) Add an additional 30uL of uncolored sample buffer (diluent) to **First** Reference P/N well.
IMPORTANT: The last sample added on each plate must be a 2nd set of controls. Therefore, if you fill an entire plate, row H columns 11 &12 must contain Reference P/N. If you fill only half a plate, the last sample you add must be Reference P/N.

3) Add **200uL Uncolored sample buffer (Diluent)** to each well for a **Sera sample (columns 7-12)**
Note: the last P/N well will now have 200uL, which is correct for this well.

4) Vortex and add 20uL of diluted Reference P/N to wells A1 (measles/rubella virus well) and A2 (cell control well). Repeat for the last pair of wells on the plate (such as H11 and H12 if you use an entire plate).

5) Add **50uL of the first DBS eluate sample from the prepared 96 well plate** to columns 1 & 2 of row B. Repeat for other samples by adding 1 sample per well to columns 1 & 2, 3 & 4, 5 & 6.

The plate set-up will look as follows:

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	Ref P/N	Ref P/N	8	8	16	16	24	24	32	32	40	40
B	Sample 1	Sample 1	9	9	17	17	25	25	33	33	41	41
C	Sample 2	Sample 2	10	10	18	18	26	26	34	34	42	42
D	3	3	11	11	19	19	27	27	35	35	43	43
E	4	4	12	12	20	20	28	28	36	36	44	44
F	5	5	13	13	21	21	29	29	37	37	45	45
G	6	6	14	14	22	22	30	30	38	38	46	46
H	7	7	15	16	23	23	31	31	39	39	Ref P/N	Ref P/N

6) Add **20uL of the first diluted sera sample from the prepared 96 well plate** to columns 7 & 8 of row A. Repeat for other samples by adding 1 sample per well to columns 9 & 10, 11 & 12.

5) Cover with clear plate sealer and **incubate 60 minutes @ 37°C & 5% CO_2**. (During this incubation, cover and place 96 well plates containing extracted patient sample and milk elution buffer @ 4°C if needed for another assay, or @ -20°C or -80°C if the current assay completes both measles and rubella testing.)

6) **Wash 5 times with 400uL wash solution**:

a.) Dump contents of plate in sink and tap plate on paper towel to remove excess liquid (antibodies have adhered to antigen on plate so you won't lose them!).

b.) Quickly add 400uL of wash solution per well with a multichannel pipette to all wells (you may have to add 200uL twice). Do this quickly so wells do not dry out!

c.) Repeat 3 times for a total of 5 washes.

7) **Add 100uL GREEN α-human IgG/ Conjugate** per well with a multichannel pipette to all wells.

8) Cover with clear plate sealer and **incubate 60 minutes @ 37°C & 5% CO_2**. (During this incubation, make Chromogen Solution in a new 15mL conical tube. Do not use the bottle provided in the Supplementary Reagents kit.)

a) Working Chromogen Solution = 1mL Chromogen TMB + 10mL Substrate TMB
- LIGHT SENSITIVE - wrap with foil and put in the dark!!!

9) **Wash 5 times** as above.

10) **Add 100uL Working Chromogen Solution** per well.

11) Cover and **incubate 30 minutes @ room temperature IN THE DARK** (put in a drawer).

12) DO NOT WASH!!! **Add 100uL stop solution** per well.

13) **Read optical density** (OD) on plate reader (450nM).

14) Dispose of plate, working solutions, and any waste in biohazard containers. Place remaining ELISA plates and reagents in refrigerator.

CALCULATION OF RESULTS

1) Open Excel spreadsheet for Measles OR Rubella test results.
2) Copy the original worksheet to the end of the Excel sheet and rename with today's date.
3) Follow instructions on worksheet.
4) Write the date on the kit insert containing the lot #, nominal value, alpha and beta and scan into the computer.
5) Email the updated Excel spreadsheet, scanned kit insert, and plate reader's Excel spreadsheet containing original OD values to: