

RECOMMENDATIONS FOR MINK BLOOD SAMPLING FOR DIA

1. Dot immunoenzyme assay (DIA) is a method for detection of Aleutian disease virus-specific antibodies in mink blood samples applied onto nitrocellulose membranes. Upon completion of this assay, positive signals develop as colored dots on the membrane. DIA ensures minimal risk of sampling errors in mass screening compared to other methods, such as CIEP or ELISA. This is because the reaction is developed directly on the membrane used for sampling. Because of minute amounts (0.5-1 microliter) of whole blood required for testing, the method obviates the need for capillary tubes. Instead, standard disposable lancets¹ made of stainless steel are used for blood sampling and spotting onto the membranes. This approach dramatically saves sampling time and excludes mink bleeding.
2. On the day of blood collection, do not feed minks before sampling to avoid fat in the blood.
3. Use provided nitrocellulose membranes for blood sampling. Each kit contains 10 membranes. Each membrane has a grid for 200 samples. The membranes are supplied with waterproof separation sheets attached underneath. Never remove separation sheets from the membranes, as this may cause cross-contamination among membranes.
4. Fix membrane (together with separation sheet) by pins (or by lancets) on any clean and even surface. Mark the membrane at the margins using ball pen to identify samples (date of sampling or shed # or mink #). Use gloves. Do not touch the grid area with ungloved hands to avoid leaving fingerprints on the membrane.
5. Fix mink properly to provide access to one of its paws. Puncture mink finger pad with sharp end of a disposable stainless steel blood sampling lancet.



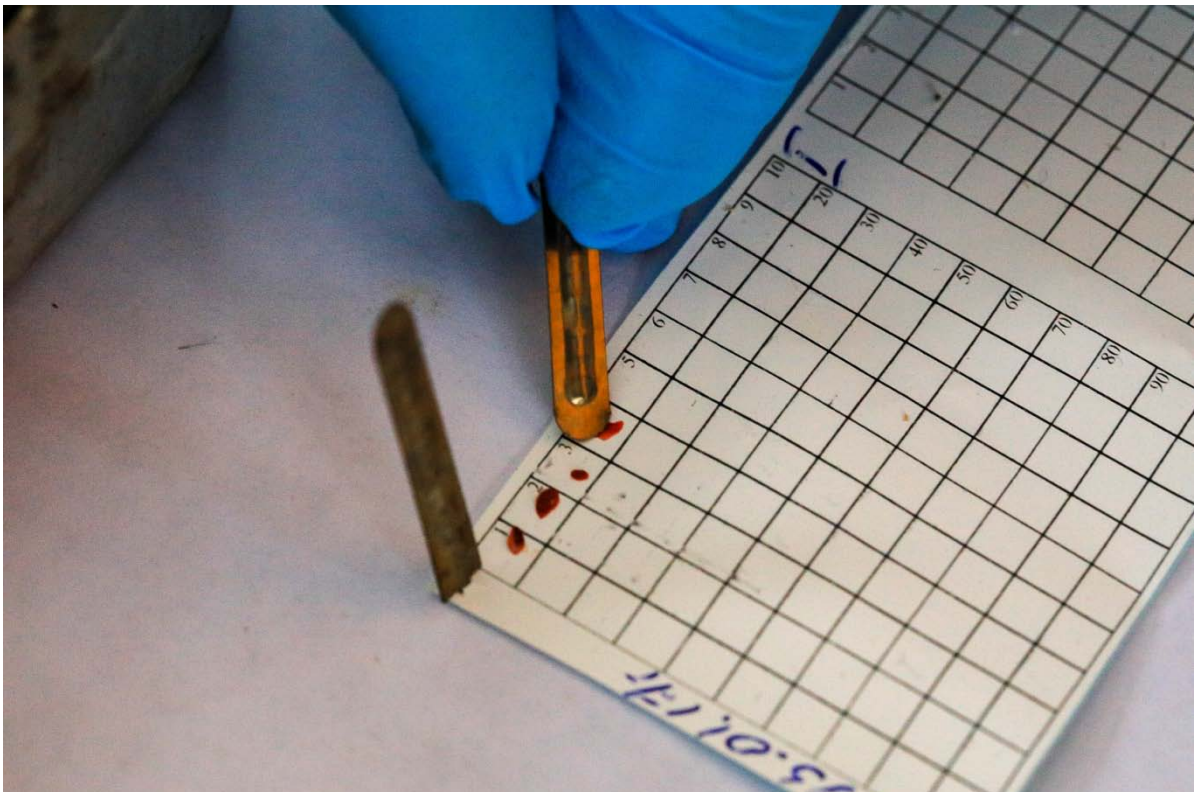


6. Wait a second until a drop of blood appears. If no bleeding, massage the finger lightly. If massaging does not help, puncture another finger pad.



¹ The lancets are not included into the DIA kits. There are a number of lancet manufacturers in Europe, China and India. Select lancets with round blunt end for mink blood sampling for DIA

7. Pick up a drop of blood using blunt end of the lancet and apply it onto the membrane. Lancets with a round blunt end are preferable to prevent membrane damage. Avoid spreading of the blood spot beyond the borders of the individual cell on the membrane. Make sure that blood samples are dry before putting the membranes together.

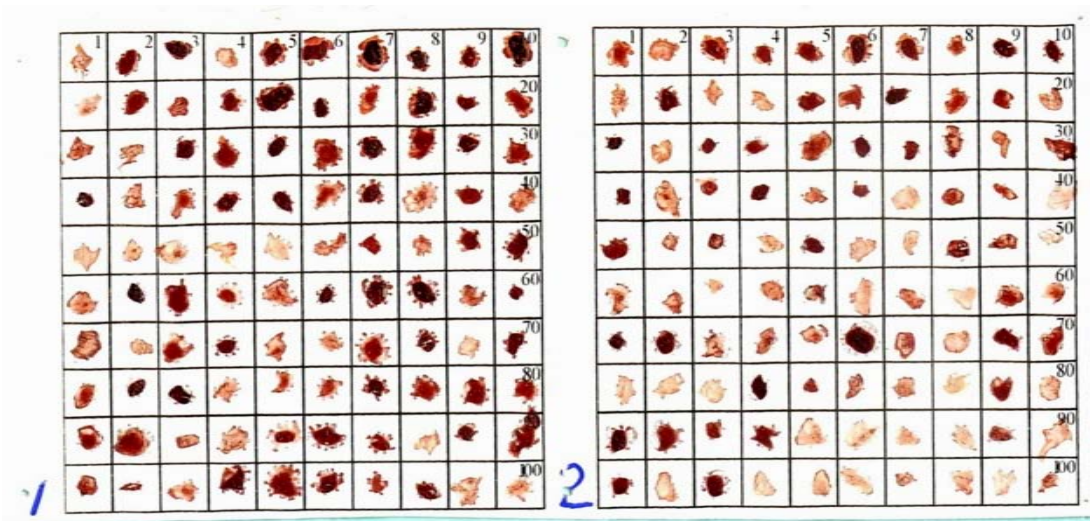


8. Use alcohol-wetted cotton balls to clean and disinfect gloves periodically. Be careful. Avoid blood contamination by the disinfectant.

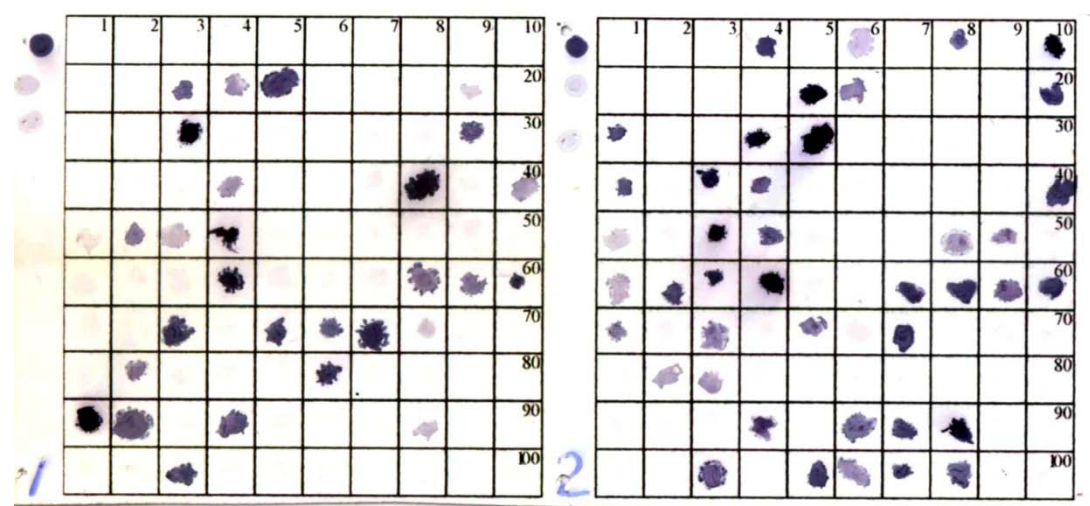


9. Put the set of membranes (up to 200) into a small package and send to the lab by express mail. The DIA procedure tolerates up to three weeks of time between blood sampling and processing. However, it is strongly recommended to send membranes with samples to the lab as soon as possible. Keep in mind that intensity of positive signal starts to fade gradually after a week of storage.
10. Shown below is a membrane before and after DIA processing. Positive reactions appear as violet spots of varying intensity. Spots on the margins are positive controls (one strong and two weak) applied before processing.

BEFORE



AFTER



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