



EURL-AP Standard Operating Procedure

Slide preparation and mounting

Experts for edition and revision	
Version 1.0	Last major revision
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1. SCOPE AND PURPOSE

The purpose of the SOP is to present how slides shall be prepared and mounted for observations by light microscopy. **This SOP is a binding complement to point 2.1.4.1 of Annex VI to Commission Regulation (EC) No 152/2009 as lastly amended by Commission Regulation (EU) No 51/2013.**

2. SUMMARY

This SOP explains how to prepare and how to mount slides that will be used for the observations by light microscopy for the detection of constituents of animal origin in feed materials and compound feed. It describes two types of slide preparations: non-permanent (or wet mounts) and permanent.

3. VALIDATION STATUS AND PERFORMANCE CHARACTERISTICS

NA

4. DEFINITIONS

Abbreviations used :

- SOP : standard operating procedure
- NA : not applicable
- NOA65 : Norland ® Optical Adhesive 65

5. HEALTH AND SAFETY WARNINGS

NOA65 should be handled with care. Material Safety Data Sheet should be read for this product as well as for any associated products such as alcohol, acetone or methylene chloride. Prolonged contact with skin should be avoided and affected areas should be thoroughly washed with copious amounts of soap and water. If NOA65 gets into the eyes, flush with water for 15 minutes and seek medical attention. Use the resin in a well-ventilated area.

NOA65 is polymerized by UV light. Cautions related to eye exposure to UV light should be taken.

6. EQUIPMENT AND MATERIALS

- Mounting media: described in point 2.1.2.1.3. of Annex VI to Regulation (EC) No 152/2009.
- Classical microscope slides and hollow slides (for >0.25 mm fractions)
- Coverslips (20x20 mm)
- Tweezers
- Fine spatula
- UV source (λ 350-380 nm)

- UV protective glasses
- Gloves

7. STEP BY STEP PROCEDURE

7.1. Sample preparation

Representative test portions of different fractions (sediment, flotata or raw material) are prepared in accordance with point 2.1.3.3. of Annex VI to Regulation (EC) No 152/2009.

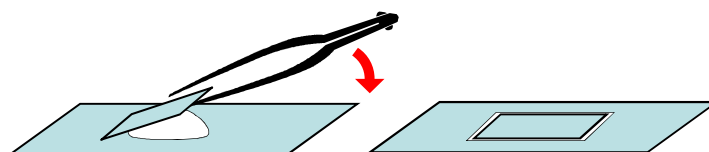
In case 0.25 mm sieving of fractions was performed use classical slides for the ≤ 0.25 mm fractions and hollow slides for the > 0.25 mm fractions.

7.2. Non-permanent slide preparation

- Place a clean slide on the desk.
- Pour carefully some drops of chosen mounting medium on the slide.

AVOID THE FORMATION OF AIR BUBBLES.

- With the spatula put some test portion of a fraction (e.g. ~10 mg from sediment, flotata or raw material) on the mounting medium. Stir gently in order to spread uniformly the material.
- Cover with coverslip: hold it with tweezers and lay it carefully down on the mounting medium deposit (cf. illustration below).



- In order to keep non-permanent slides observable for a longer period, up to several days, sealing of the edges of the coverslip can be realised. Sealing can be performed by applying a thin layer of nail varnish or valap (mixture of equal quantities w/w of vaseline, lanolin and paraffin wax).

7.3. Permanent slide preparation

SWITCH ON UV SOURCE BEFORE STARTING, AND ALWAYS WEAR UV PROTECTIVE GLASSES.

- Place a slide on the desk.
- Pour carefully some drops of NOA65 on the slide (5 drops for classical slides – 7 drops for hollow slides).

AVOID THE FORMATION OF AIR BUBBLES.

- Close the resin vial again and keep it away from any UV source.
- With the spatula put some test portion of a fraction (e.g. ~10 mg from sediment, flotata or raw material) on the resin. Stir gently in order to spread uniformly the material over the resin deposit.

*FROM NOW ON YOU GOT ABOUT **1 MIN** BEFORE POLYMERIZING.*

Slide preparation and mounting

- Take a coverslip, hold it with tweezers and lay it carefully down on the resin deposit (cf. illustration on page 3).
- Allow the resin to spread all under the coverslip surface (for hollow slides you may have to press very carefully on the coverslip in order to achieve this more rapidly).
- Put the slide under the UV source (at a distance of about 3 cm from the tube). Allow to polymerise for at least 2 min (longer time is preferred but not mandatory knowing that total polymerisation takes about 20 min. Nevertheless satisfying hardening is yet obtained after some 30 sec).
- Remove slide from the UV source.

8. INTERPRETATION OF RESULTS

NA

9. REFERENCES

Commission Regulation (EU) No 51/2013 of 16 January 2013 amending Regulation (EC) No 152/2009 as regards the methods of analysis for the determination of constituents of animal origin for the official control of feed. Official Journal of the European Union, L20, 23.1.2013, p.33-43.

Version	Publication date	Application date	Repeal date
1.0	28.03.2013	28.04.2013	