Date: 9 July 1999

To: All Holders of Manual 10A

Manual 10A: The Sulpha-on-Site Test - Issue 3

Manual 10A: The Sulpha-on-Site Test has been revised to amend to requirements of proficiency testing to incorporate performance based principles. In addition, the opportunity has been taken to enhance the procedures for performing the test.

Attached are **update** pages for your copy of the above document

Remove all old pages	**Insert all new pages**

Please **sign-off and date** the Amendment Record.

Amendment authorised by:

Tony Zohrab
Director (Animal Products)
MAF Food Assurance Authority (Animal Products)

Manual 10A

The Sulpha-On-Site Test

Procedures for the Screening of Calf Plasma for Sulphonamide Drug Residues

MAF Food Assurance Authority (Animal Products)
Wellington

Issue 3, July 1999

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Acknowledgments

MAF Food Assurance Authority gratefully acknowledges the technical input provided by Mike Clear and Carmen Saba of the National Chemical Residue Laboratory in the publication of this document.

Amendment Record

Amendments are given a consecutive number and dated.

Please ensure that all amendments are inserted and complete the record below.

Issue No.	Date	Initials	Issue No.	Date	Initials
2	June 1998	Incorporated			
3	July 1999	Incorporated			

Distribution

Manual 10A has been published as the MAF Food Assurance Authority-approved method for the on-site testing of bobby calves.

It has been distributed to the MAF Verification Agency at all premises which have processed bobby calves in the past. The MAF Verification Agency at any premises which intends to process bobby calves in the future shall obtain a copy of Manual 10A. It is available

- In electronic form, from the MAF Website at:

  www.maf.govt.nz/meatdoc/meatman/man10a/httoc.htm

- In hard copy, from:

  Publishing Solutions Limited
  P.O. Box 983
  Wellington

  Phone: (04) 471 0582
  Fax: (04) 471 0717

Contents

1. SOS Testing Competency

1.1 General principle

1.1.1 To ensure all personnel conducting SOS testing on regulatory samples are appropriately trained, and achieve and maintain competency to perform the test.

1.2 Laboratories approved to provide training and proficiency testing programmes

1.2.1 Training courses and proficiency testing programmes can only be provided by MAF Food Assurance Authority-approved laboratories. The MAF Food Assurance Authority-approved laboratory is:

- National Chemical Residue Laboratory (NCRL), AgriQuality NZ.

1.2.2 Training and proficiency testing programmes are co-ordinated by the NCRL SOS Programme Co-ordinator. This person is also responsible for assessing test results received from individuals participating in any of the proficiency test rounds. All operational enquires regarding training or proficiency testing should be directed to this person.

1.3 Proficiency testing

1.3.1 Proficiency testing provides an independent assessment of an individual's ability to competently perform the SOS test.

1.3.2 SOS proficiency test samples are prepared in a laboratory either by spiking bobby calf plasma samples with known concentrations of sulphonamide compounds, or leaving the plasma negative. These samples are uniquely identified by code which is known only to laboratory staff involved in the proficiency testing programme. Individuals are required to analyse the samples using the same procedures as those applied to regulatory samples, and to report their results back to the laboratory.

1.3.3 In the SOS proficiency testing programme, there are five types of proficiency test samples:

- Training samples — required to be correctly reported before an individual can be certified as competent.

- Pre-season proficiency test samples — for those individuals with a higher level competency at the conclusion of the previous season (refer 1.7.1). Pre-season

proficiency test samples must be correctly reported before the individual re-commences testing regulatory samples.

- Mid season proficiency test samples — for higher level competent individuals who qualify by having gained an acceptable result in the pre-season proficiency round (refer 1.7.1). These samples are despatched at the median time of the national bobby calf kill season.

- Programmed monthly proficiency test samples — for entry level competent individuals in their first season following training, and for those individuals whose performance requires further assessment prior to regaining higher level competency (refer 1.6.2).

- Customised proficiency test samples — individualised samples sent to an individual who has not performed as required in a pre-season, programmed monthly or mid-season proficiency testing round, or for any other reason as appropriate.

1.4 Principles of the proficiency testing programme

1.4.1 The SOS Proficiency Testing Programme applies performance-based principles in which criteria are designed to:

- establish and maintain competency,
- state performance standards required to be met so that minimal proficiency testing can apply,
- establish actions required to correct performance failures.

1.5 Competency of individuals performing the SOS test

1.5.1 SOS testing on regulatory samples shall only be performed by individuals who have been trained by an approved training provider and have been assessed as competent to conduct the testing.

1.5.2 Individuals will be certificated competent when they have:

- satisfactorily completed an approved training course, and
- demonstrated proficiency in reporting a set of training samples.

1.5.3 These individuals have **entry level competency**. Progression to a **higher level competency** is assessed on the basis of satisfactory performance in the SOS Proficiency Testing Programme throughout a bobby calf season.

1.5.4 Table 1 and Sections 1.6-1.8 describe the proficiency testing requirements for progression and maintenance to higher level competency status, and also describe the actions required to correct performance failures.

1.6 Entry level competency

1.6.1 Individuals who have satisfactorily completed an approved training course and reported a set of training samples with an acceptable result are deemed competent at the **entry level** and may undertake SOS testing of regulatory samples.

1.6.2 Individuals with **entry level** competency shall participate in a minimum of **two** programmed monthly proficiency testing rounds during the bobby calf season. Approximately four programmed monthly proficiency testing rounds will be available throughout a season. It is up to the individual to elect the two rounds in which they will participate. It is expected that the two rounds will be undertaken within the bobby calf season for that premises where the individual is based.

1.6.3 Upon successful completion of two programmed monthly proficiency testing rounds, an individual will attain **higher level** competency, which will be carried over to the commencement of the next season.

1.7 Higher level competency

1.7.1 Individuals carrying over **higher level** competency status from the previous season shall participate in a pre-season proficiency testing round. If successfully reported, further participation in a mid-season proficiency testing round shall be undertaken.

1.7.2 Upon successful completion of the mid-season proficiency testing round, an individual will be deemed to have maintained the **higher level** competency status which will be carried over to the following season.

1.8 Procedures which are implemented if an individual fails the proficiency testing rounds

1.8.1 Upon receiving an unsatisfactory result for any of the programmed monthly, pre-season or mid-season proficiency round test samples, the individual shall cease testing regulatory samples.

1.8.2 A set of customised proficiency test samples will be provided by the laboratory which shall be analysed and reported. If the results are acceptable, the individual may resume testing of regulatory samples, and shall then complete at least **two** monthly proficiency rounds for the remainder of the season.

1.8.3 If the results are unacceptable, an investigation will be undertaken in conjunction with the laboratory and a second customised set of samples will be provided for analysis and reporting. If the results are acceptable, the individual may resume testing regulatory samples. The individual shall then complete at least **two** monthly proficiency rounds for the remainder of the season.

1.8.4 Failure with two customised proficiency testing rounds will result in the individual being deemed no longer competent to perform SOS testing.

1.9 Actions for individuals deemed not competent

1.9.1 An individual is deemed to be no longer competent to perform SOS testing when:

- they have failed successive customised proficiency tests (see Section 1.8.4)
- they have not retained competency from the previous season.

In such instances, the individual will require re-training.

1.9.2 Once they have been re-trained and they have demonstrated competency with a set of training proficiency test samples, the individual will gain **entry level** competency. The requirements of Section 1.6. then apply.

1.10 Schedule of proficiency testing throughout a season

1.10.1 MAF Food Assurance Authority will advise a schedule of dates throughout the bobby calf season that pre-season, programmed monthly and mid-season proficiency samples will be despatched. The schedule will be based on the previously supplied notification of the slaughter season start and finish dates.

1.10.2 The Technical Supervisor (TS) shall notify the laboratory which individuals will be participating in the pre-season, nominated monthly or mid-season proficiency testing rounds. In as much as possible a schedule for each participating individual for the entire season shall be provided prior to the commencement of the bobby calf season. In the event that a full season of participation cannot be anticipated for an individual, the TS shall notify the laboratory at least one week prior to despatch of any proficiency test samples which individual(s) will be taking part in that testing round.

The TS shall also advise which organisation each individual is employed by.

1.10.3 All proficiency testing rounds, with the exception of training samples, comprise a set of three plasma samples for analysis. Training samples are received as a set of six samples.

1.11 All proficiency test samples

1.11.1 The TS shall notify the laboratory when proficiency test samples have been received at the premises (problems with couriers in the past have resulted in non-delivery).

1.11.2 Individuals shall perform SOS testing in accordance with the protocol in this manual, and shall use exactly the same procedures to analyse their samples as those applied to regulatory samples.

1.11.3 Samples should be refrigerated on receipt. If the samples are unable to be analysed within 24 hours of receipt, they should be stored in a freezer prior to analysis.

1.11.4 The TLC plate shall be viewed only by the individual those samples were assigned to, and reported to the laboratory without consultation with other people.

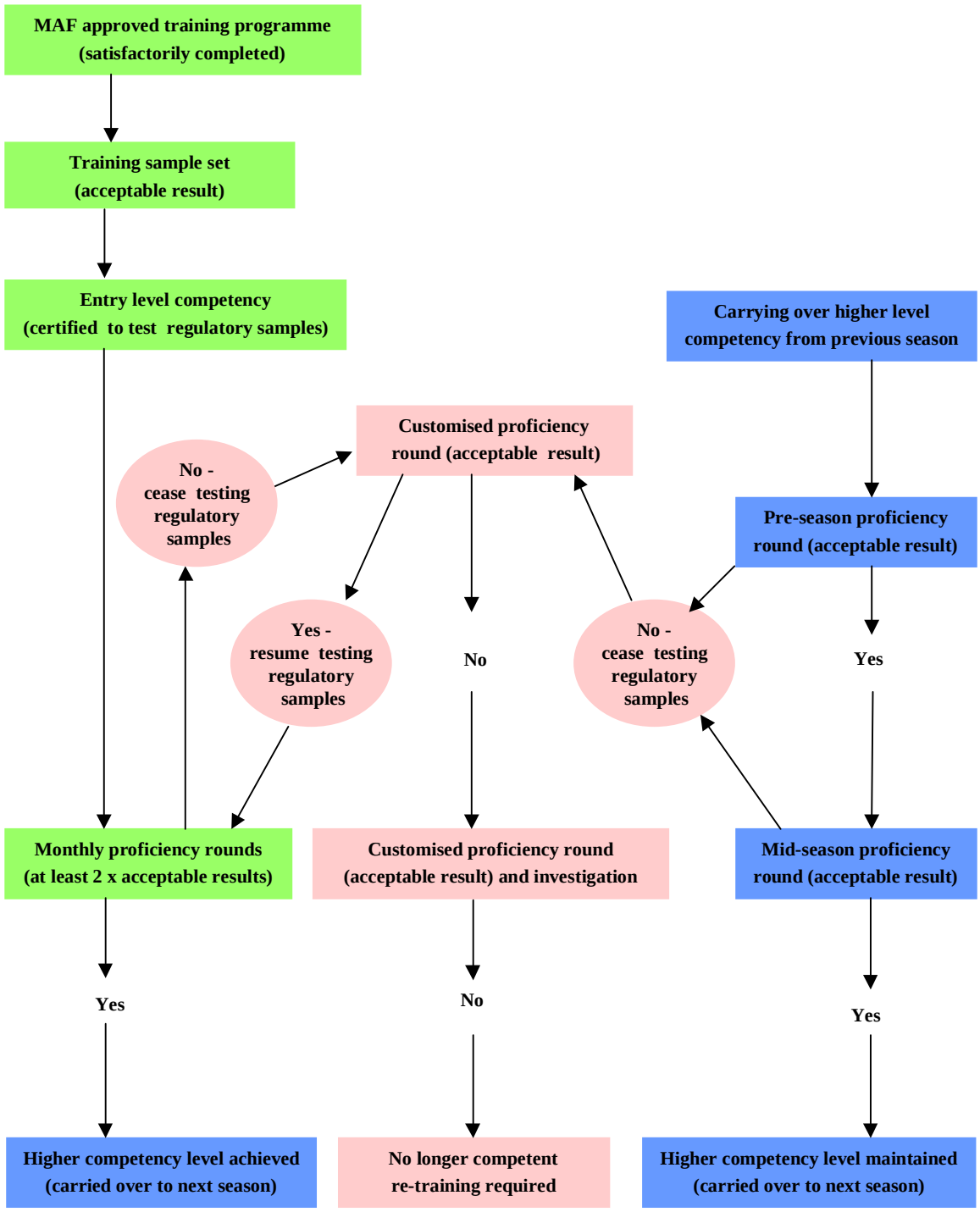
1.11.5 The samples shall be analysed and results faxed back to the laboratory within 5 working days of receipt of the samples at the premises.

1.11.6 The results shall be recorded in pen on the result form supplied with the samples. These results shall be sent to the laboratory by **fax** immediately following completion of the test.

1.11.7 The TS shall ensure that each certificated individual analyses a set of samples in accordance with the requirements of the proficiency testing programme. The laboratory will send the TS a summary of the individual's results.

1.11.8 The TS shall be responsible for maintaining a register of current competent individuals, and ensure that only those individuals perform SOS testing on regulatory samples.

Table 1 Decision Tree for Competency Levels



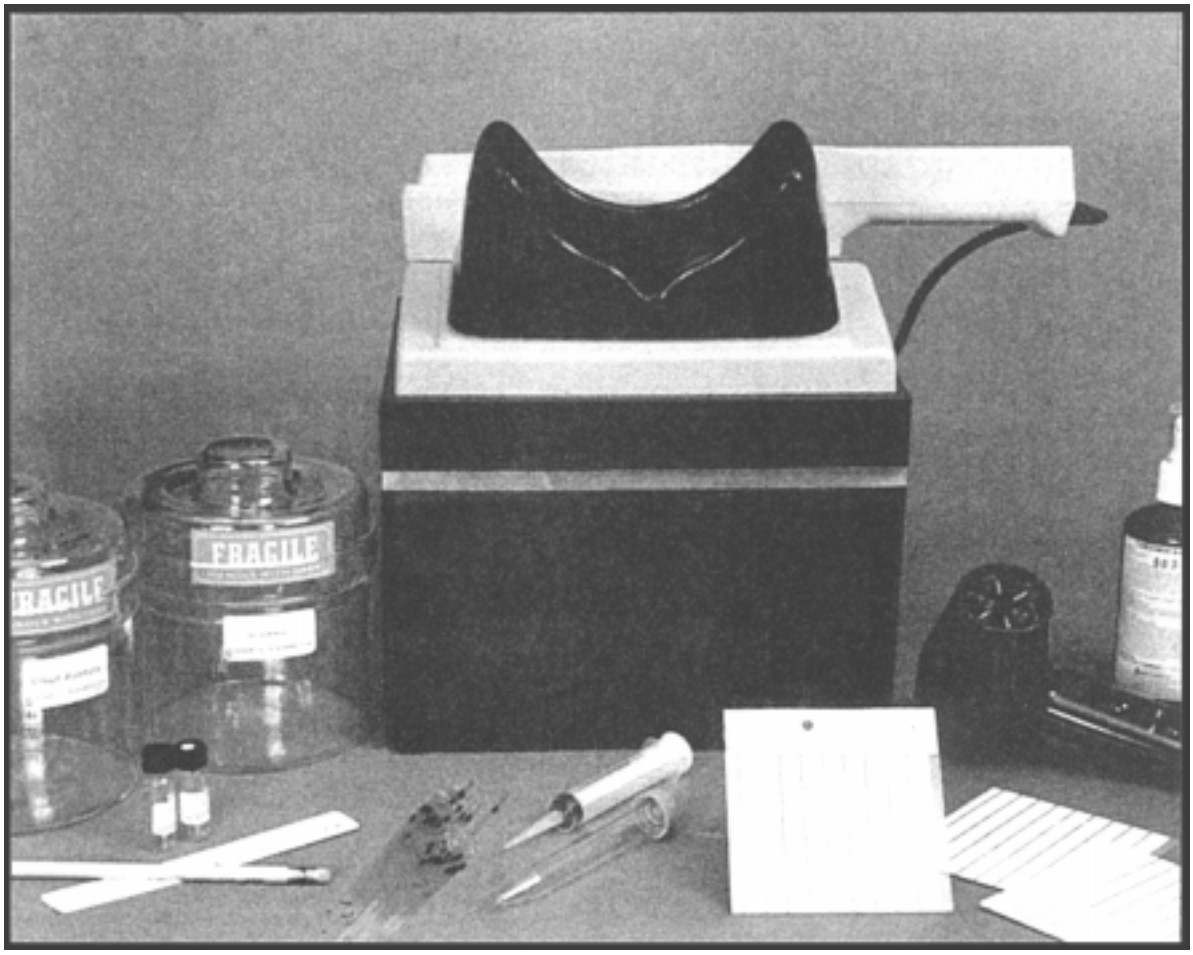
2. Objectives of the Test

The purpose of this test is to analyse plasma from selected bobby calves by thin layer chromatography (TLC), and to detect any residues of sulphonamide drugs by spraying the TLC plate with a solution of fluorescamine in acetone. There are a number of different sulphonamide drugs licenced in New Zealand for veterinary use, but none are licensed for use in bobby calves. The test is particularly sensitive to sulphamethazine, and this compound is used as the reference material in this test. These procedures will also explain how you can determine if another sulphonamide is present.

3. Contents of the Kit

3.1 Durables

- 1 Ultraviolet (u.v.) viewing box
- 1 Ultraviolet (u.v.) lamp-long wavelength (360 nm)
- 1 Blow dryer (any hair dryer with a warm setting will do)
- 1 Chromatography tank labelled “Methanol”
- 1 Chromatography tank labelled “Ethyl Acetate” (any glass jar with a lid and large enough to hold the plates may be used as an alternative)
- 1 Wooden block with holes to hold standards (make in a workshop)
- 1 Test tube rack (make in a workshop as needed)
- 1 Soft lead pencil (obtain replacements from a stationer)
- 1 Plastic ruler (obtain replacements from a stationer)
- 1 Spray pump (replace with a perfume sprayer from a pharmacy)
- 1 50 ml polypropylene falcon tube (to be used for measuring 30 ml of acetone)
- 1 Wide-necked amber polypropylene bottle with a screw cap lid
- A centrifuge capable of at least 2000 rpm and large enough to hold at least four 10 ml vacutainer tubes.



3.2 Replacement items

- Acetone
- Fluorescamine dry powder, 10 mg
- Methanol
- Ethyl acetate
- Thin layer chromatography plates: "Whatman" 10 cm × 10 cm, 250 µm layer LK6D
- Capillary pipettes
- Polystyrene centrifuge tubes
- Sulphamethazine standard: 0.4 µg/ml
- 3 ml graduated plastic transfer pipettes
- 10 ml heparinised vacutainer tubes (green top)
- Vacutainer needles
- Blotting paper (obtain replacements from a stationer)

4. Health and Safety

The provisions of the Health and Safety in Employment Act 1992 must be complied with in respect of this test.

Refer to the safety sheets which are contained in the kit for details of the hazards associated with particular test components.

Particular hazards are:

Methanol	Toxic by ingestion, inhalation or skin absorption. Flammable liquid vapour. Keep away from ignition sources and acids. Store methanol in a fireproof box at ground level.
Ethyl acetate	Skin irritant. **Inhalation hazard**. Flammable liquid and vapour. Keep away from ignition sources and acids. Store ethyl acetate in a fireproof box at ground level.
Acetone spray	Skin irritant. **Inhalation hazard**. Avoid breathing vapours. Highly flammable liquid and explosive vapour (spray). Keep away from ignition sources. Store in a fireproof cabinet.
Centrifuge	Do not open the centrifuge lid while the rotor is still moving. Ensure the rotor is balanced before operating.
Ultraviolet light	Harmful to the eyes. Generates ozone. Use in a ventilated area.
Fluorescamine	No known hazard, but handle solid with care.

Symptoms of excessive inhalation exposure to acetone and ethyl acetate are:

- fatigue, excitement, headaches, bronchial irritation;
- in large amounts, narcosis.

Pipetting by mouth is not permitted under any circumstances. Devices are available to assist in this operation.

5. Precautions and Care of Reagents

5.1 Solvents

Do not tip waste solvent down the sink drains; discard it to a waste bottle or allow it to evaporate outside. Residual solvent remaining in the TLC tanks after analysis of a batch must not be returned to the stock bottles. The TLC tanks are not airtight and the residual solvent should be either safely disposed of or stored in a suitable airtight container for reuse with the next batch. The TLC tanks are filled with the recycled solvent and topped up from the stock bottles of solvent if necessary.

5.2 Fluorescamine

Fluorescamine is supplied as a dry powder in a sealed vial and dissolved in acetone when required. The prepared fluorescamine solution should be stored in an amber-coloured wide-necked plastic bottle in a dark cool cupboard. The fluorescamine solution, once prepared from solid, is stable for 1 month. The fluorescamine solution should not be used beyond its expiry date. Provided it is kept dry, the dry powder is stable for longer than 1 month.

Transfer only the required volume for the sample batch to the spray device. Unused reagent can be returned to the storage bottle.

Do not allow the fluorescamine solution or the spray device to come into contact with water. Fluorescamine reacts instantly with water and the solution will become ineffective, resulting in a failure of the test. If this occurs, a new fluorescamine solution in acetone needs to be prepared. Using fresh solutions of fluorescamine will eliminate one potential source of analytical error.

5.3 Sulphonamide standard

Sulphamethazine is supplied dry in screw-capped vials. The quantity of drug is too small to be visible. The drug is rendered soluble by dissolving in methanol with vigorous shaking. When not in use, the solution should be stored in a cool dark cupboard with the lid tightly sealed. During laboratory operations the lid should be replaced immediately after use to minimise evaporation. The sulphamethazine standard, once made up into a solution with methanol, is stable for 1 month. The standard solution should not be used beyond the 1-month expiry date.

5.4 TLC plates

Consistent performance from the TLC plates depends on freedom from extraneous contamination, particularly water as either liquid or vapour. Unused plates should be stored in a covered container away from vapour or smoke and preferably where the

temperature remains stable. Storage at temperatures below that of the laboratory will cause condensation on the plate when exposed and give poor chromatographic resolution. Every precaution should be taken to avoid touching the absorbent material on the TLC plates.

If only a few samples need to be analysed, the use of partial plates is permissible. A plate may be scored with a glass knife along one of the lane divisions and broken. If the break does not occur cleanly along the divider, that lane should not be used.

5.5 Test sensitivity

It is desirable to operate the test at maximum sensitivity; the most critical element in achieving this is the size of the spotting circles. These can be kept small by judicious use of the hair dryer between the 20 µl applications and application technique. Any sulphonamide in the sample runs to the outside circumference of the spotting circles, and, if they are large, any sulphonamide remains at the extreme edge of the silica lane where it becomes difficult to see.

5.6 Plasma

If the analysis is not to be started immediately, the plasma samples may be stored in the refrigerator for up to 24 hours.

6. Scientific Basis of the Test

The test is an application of thin layer chromatography (TLC). A TLC plate has a number of lanes made of adsorbent material, in this case specially refined silica of very small particle size. The lower part of the plate is thicker than the rest of the lane and is called the loading zone. Any compound spotted on to the loading zone which is partially soluble in the eluting solvent will migrate up the lane according to the eluting power of the solvent. Methanol is a strong eluter and ethyl acetate somewhat weaker.

When the solvent has moved a certain distance up the plate, the plate is removed from the solvent and residual solvent removed by drying with hot air. The evaporation of the solvent fixes the position of a compound on the plate and this position is a characteristic of that compound. The plate is then sprayed with a solution of fluorescamine (in acetone) which reacts with part of the sulphonamide drug molecule to form a new fluorescent molecule.

The fluorescent colour of green-yellow is only visible when the complex is irradiated with u.v. light of the correct wavelength.

The intensity of the band is related to the concentration of the original sulphonamide drug, and it is possible to estimate concentration if the standard and sample intensity are not very different.

The fluorescent complex is not persistent and trace levels will fade after 2-3 hours, although much higher levels may persist for up to 12 hours.

7. Performing the Test

7.1 Sample preparation

Step 1

Centrifuge a minimum of 5 ml of fresh blood at 2000 rpm for 10 minutes in the original tube. The upper fluid layer (straw coloured) is plasma.

Step 2

If the analysis is to be commenced immediately, transfer 1 ml of plasma to a centrifuge tube using a polypropylene transfer pipette.

Step 3

Add 2 ml (i.e. make up to the 3 ml mark) of methanol to the plasma, cap the tube and shake vigorously.

Step 4

Centrifuge the tube at 2000 rpm for 10 min and then place it in the test tube rack.

Step 5

Arrange each of the samples to be tested in the batch in order in the test tube rack.

Note 1

Ensure the centrifuge is balanced.

Make sure the vacutainer tube has the sample number on it.

The blood samples can be spun at speeds up to 3000 rpm, provided the centrifuge is safe and has been well balanced.

Note 2

If the samples are to be kept, store in a refrigerator for not more than 24 hours.

Label the centrifuge tube with the sample number.

Note 3

If less than 1 ml of plasma is obtained, the sample can still be used. If only 0.5 ml of plasma is obtained, add 1.0 ml of methanol. It is acceptable to use less than 1.0 ml of plasma as long as the plasma to methanol ratio is maintained at 1:2.

Extreme accuracy is not required for the volumes.

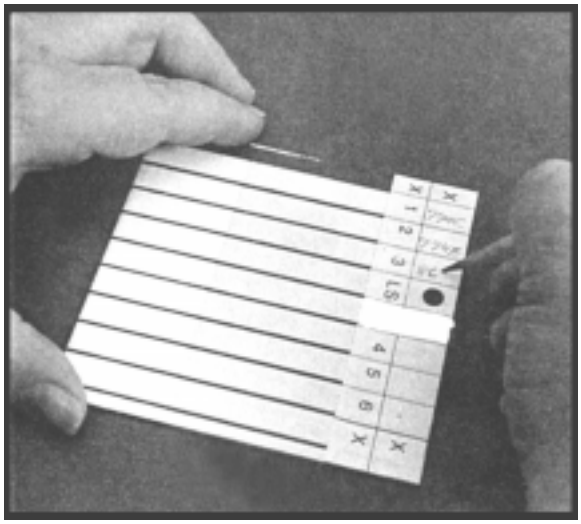
Note 4

The samples can be spun at speeds of up to 3000 rpm, provided the centrifuge is safe and has been well-balanced. Make sure the number is still legible.

About 1.0-1.5 ml of white precipitate will be seen in the bottom of the tube.

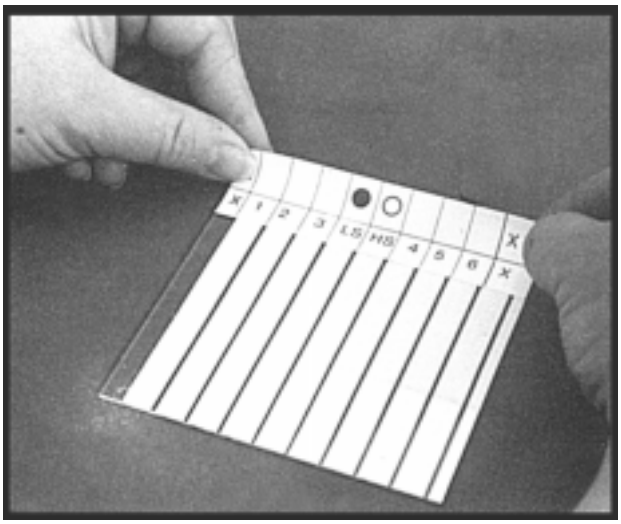
Step 6

Take a plate and draw a line 2 cm from the top. On each lane, write the sample number in pencil and leave one lane for the standard and one empty lane.



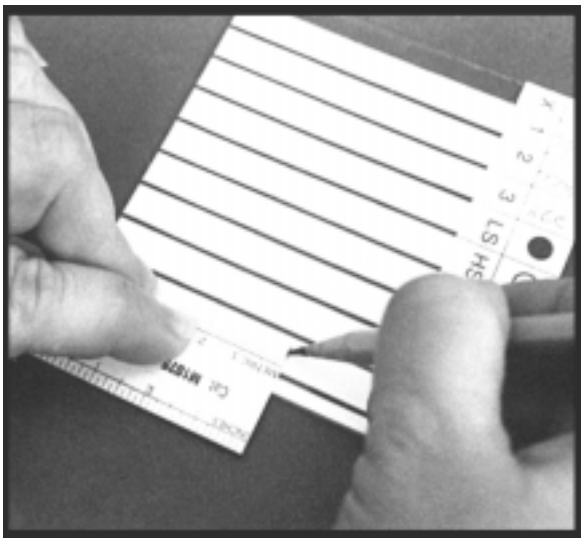
Note 6

Do not touch the plates anywhere except within the top 2 cm zone.



Step 7

Mark the empty lane 2 cm above the thick zone.



Step 8

If the sulphamethazine standard has not been used before, it will be necessary to add 2 ml of methanol to the vial using a polypropylene transfer pipette. Dissolve the standard by shaking vigorously.

Step 9

Heat the TLC place for at least 5 minutes with the hair dryer on "hot" **before** spotting the samples/standards on to the loading zone

Note 8

Store the sulphamethazine standard sealed in the dark when not in use. Ensure the standard is within the expiry date, which will be stated on the label.

It is important to dissolve all of the sulphamethazine but there is no visual end point. Shake vigorously for at least 10 minutes.

Note 9

The glass back of the TLC plate should be hot to touch. Excessive heating of the silica will not cause any damage.

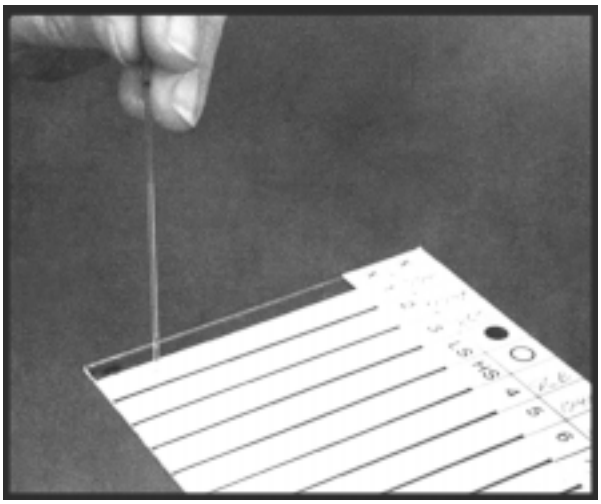
If the loading zone of the TLC plate is hot before spotting the samples, loading will be much faster, with minimal diffusion of the sample.

Step 10

Apply 20 µl of standard (0.4 μg/ml) to the correct lane and 60 µl of sample to the correctly marked lanes. Add 3 × 20 l of the blood sample to the correctly marked loading zones. Dry the lane after 40 µl has been applied, using the dryer set on "hot" for about 2 minutes. Apply a further 20 µl of plasma and dry again with the hair dryer.

The filled pipette should be applied lightly to the thick zone at a slight angle to desorb the sample on to the lane.

Use a new pipette for each sample.

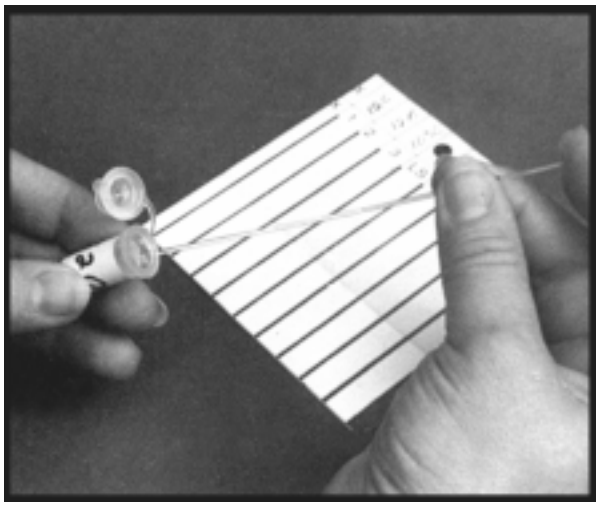


Note 10

Hold the plasma tube at a slight angle and grasp the micro-pipette between the thumb and the middle finger over the wide band. The unbanded portion holds 20 µl up to the line. Insert the tip into the sample and allow it to flow up to the line. **Do not overfill**. If too much sample is taken up, tap the tube or absorb the excess on to a tissue.

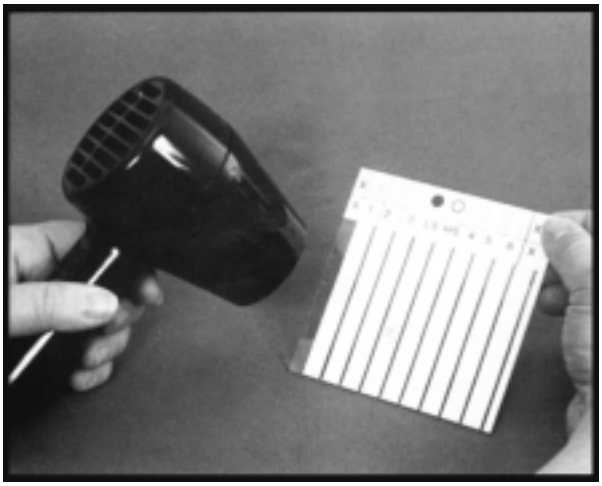
Apply the sample to the middle of the thick zone by dabbing over its entire length. Keep each dabbed area as small as possible, but not closer than 6 mm to the bottom edge. Do not damage the surface of the adsorbent. Separation and sensitivity of the test is improved greatly if the "spotting circles" are kept as small as possible.

Some of the pipettes come with plungers. DO NOT USE the plungers to fill the micro-pipettes.



Step 11

When all of the samples are spotted, dry the plate with the hair dryer set on "hot".



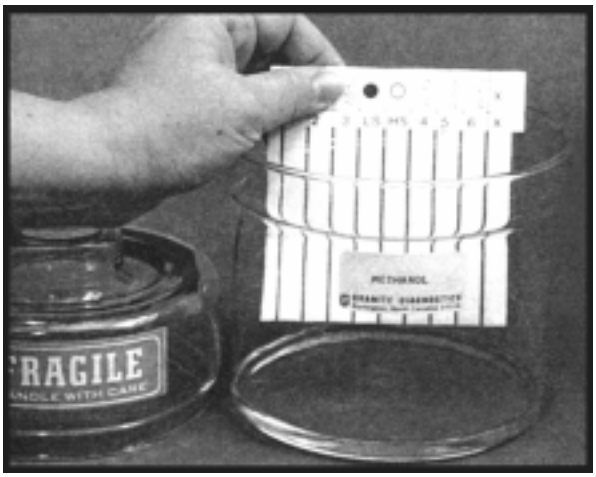
Note 11

Hold the plate at about 45° and pass the blower back and forward until the back of the plate is warm — about 2 minutes.

Step 12

Place the plate into the tank of methanol. Allow the methanol to rise to the top of the thick zone but no further.

Remove the plate and dry for about 2 minutes with the hair dryer set on "hot".



Note 12

Fill the **methanol** tank with 6 mm of methanol. If the spotting area is closer than 6 mm to the bottom edge of the plate, immerse the plate slowly and evenly until the liquid level is greater than 6 mm from the bottom. Then lower the plate to the bottom of the tank.

When drying the plate, blow the fumes away from the face. Use an "Expelair" to clear the fumes.

Step 13

Place the plate in the tank of ethyl acetate and allow the liquid to ascend to the 2 cm line level indicated in the empty lane. The minimum distance that ethyl acetate must run is 2 cm. Further than 2 cm is acceptable, provided the TLC plate is marked in pencil with the final solvent front. Remove the plate from the tank and dry with heat — about 2 minutes will suffice.

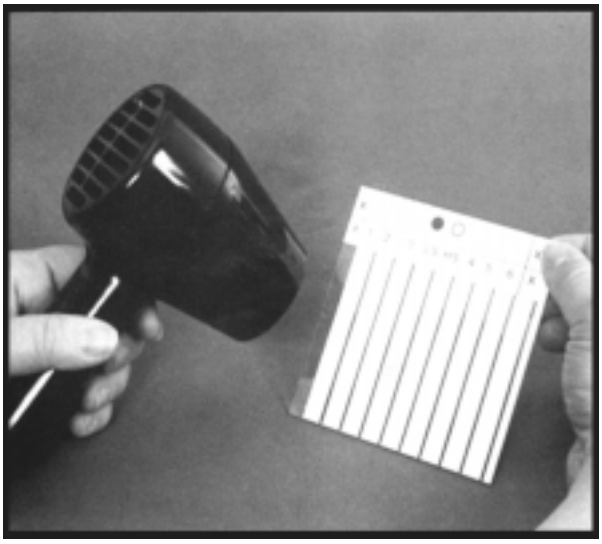
Step 14

If the fluorescamine solution has not been previously prepared, or is beyond the expiry date, prepare a new solution of fluorescamine in acetone. The dry fluorescamine powder will be supplied as a weighed quantity (10 mg) in a screw-capped vial. Transfer the dry powder into the amber wide-necked plastic bottle. Measure 30 ml of acetone into the labelled plastic conical graduated falcon tube. Rinse the glass vial with a small amount of the acetone twice, and pour the rinsings along with the remainder of the 30 ml of acetone into the amber wide-necked plastic bottle. Shake vigorously by hand.

Note 13

Fill the **ethyl acetate** tank with 6 mm of ethyl acetate. Line the rear half of the tank with blotting paper to within 2 cm of the top. Press the blotting paper to the inside surface. This improves the chromatographic efficiency.

When drying the plate, blow the fumes away from the face.

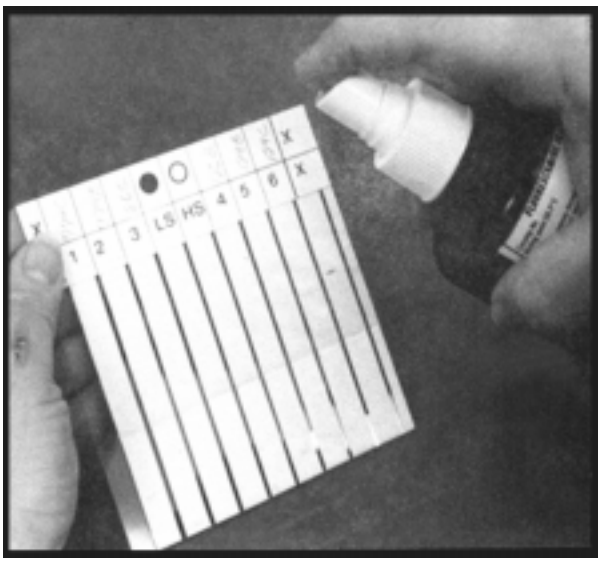


Note 14

It is not necessary to prepare a new fluorescamine solution for each batch of analysis. Store both the fluorescamine solid and the prepared fluorescamine solution in the dark, and keep dry. Fluorescamine reacts instantly with water and the solution will be ineffective if the acetone becomes contaminated with water.

Step 15

Transfer about 10-15 ml of the prepared fluorescamine solution into the spray bottle. Spray the TLC plate with the solution.



Step 16

The prepared fluorescamine solution should be stored in the amber-coloured bottle. The unused solution from the spray bottle should be returned to the amber bottle after spraying for storage.

Note 15

Hold the plate at about 45° and the sprayer about 10-15 cm away. Mist the plate evenly. Do not oversaturate the plate to the point where the fluorescamine solution runs on the plate. Do not spray the acetone when naked flames are nearby or when electrical devices (e.g. the hair dryer) are operating.

7.2 Viewing the plate

The sulphonamide standard supplied is sulphamethazine. This is only one of the many possible sulphonamide drugs that can be detected using this test. Examples of other sulphonamide drugs and their positions on the TLC plate can be seen in Figure 1. This figure shows the greenish-yellow colour that is characteristic of a positive sulphonamide. It also illustrates that not all of the sulphonamides have exactly the same position on the TLC plate as the supplied standard sulphamethazine.

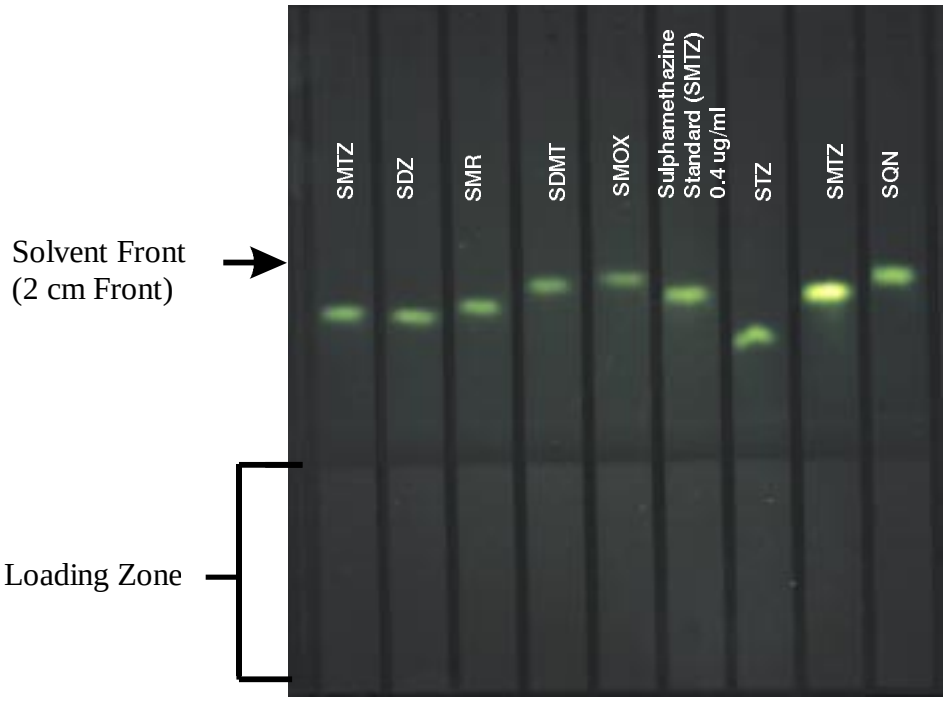
It is important to look in the area just above (as indicated in Figure 1) and just below the sulphamethazine standard, as any greenish-yellow fluorescence colour will indicate the presence of a sulphonamide.

The intensity of the fluorescence is directly related to the concentration of the sulphonamide. In Figure 2, it can be seen that as the concentration of the sulphonamide increases, so does the intensity/brightness of the greenish-yellow fluorescence.

Sample lanes may show some colour that is not the characteristic greenish-yellow colour of sulphonamides (see the sample lanes labelled “negative” in Figure 2). Other bands may be seen in sample lanes which may be blue or orange in colour. This is interference and **does not** indicate a presumptive positive sulphonamide.

In some samples, a strong fluorescent glare can be seen in the loading zone of the TLC plate. This strong greenish-yellow colour may affect the ability of the viewer to see low level fluorescence from samples. In this situation, it is advised to place a black cardboard “window” to cover the loading zone fluorescence. This can be seen in Figure 3. It is easier to view and detect any fluorescence coming from the samples.

Figure 1 Standards of Sulphonamide Drugs Commonly Used in New Zealand



Key to Abbreviations

SMTZ	Sulphamethazine
SDZ	Sulphadiazine
SMR	Sulphamerazine
SDMT	Sulphadimethoxine
SMOX	Sulphamoxazole
STZ	Sulphathiazole
SQN	Sulphaquinoxalin

Figure 2 Extracted Plasma Samples

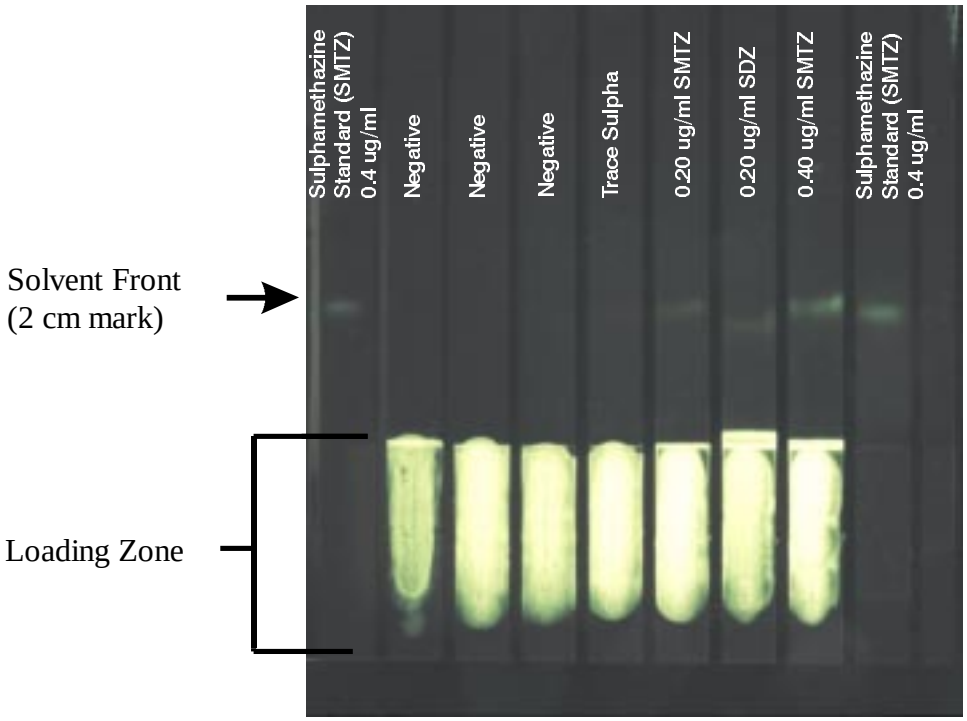
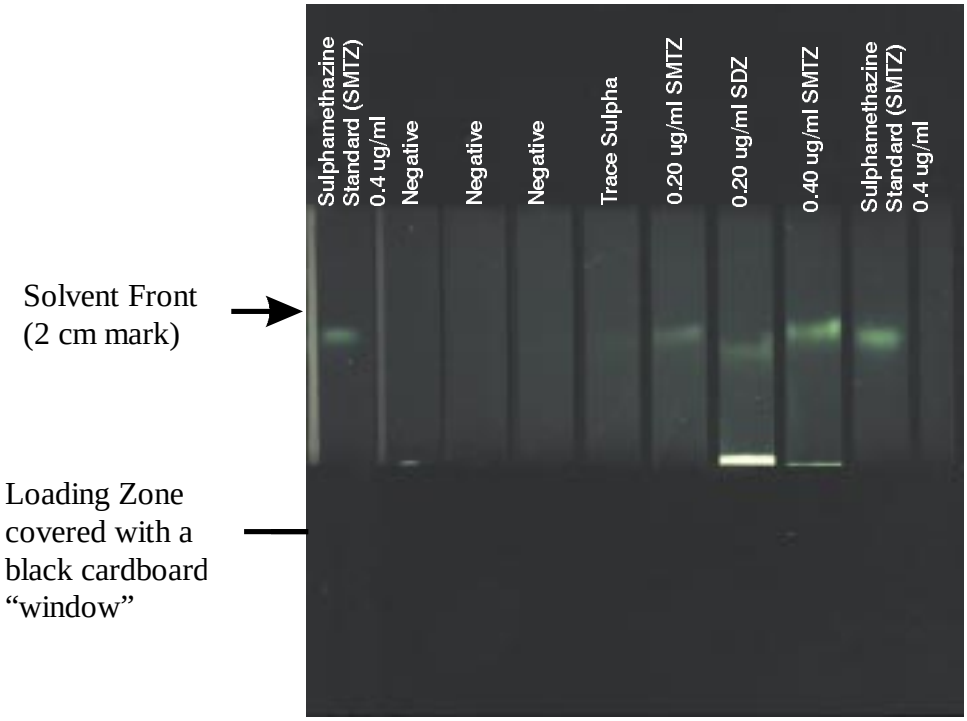


Figure 3 Extracted Plasma Samples (Loading Zone Masked)



Step 17

Lay the plate flat in the dark — in the viewing box for a minimum of 15 minutes. Maximise the contrast by viewing the plate in a darkened room and reducing fluorescent glare. Then place the lamp in position, turn it on and view the plate. Raise the plate close to the lamp and hold it at about 45^{o} and look across the lanes. A greenish-yellow colour should be visible near to, but just below the 2 cm line in the lane for the standard.

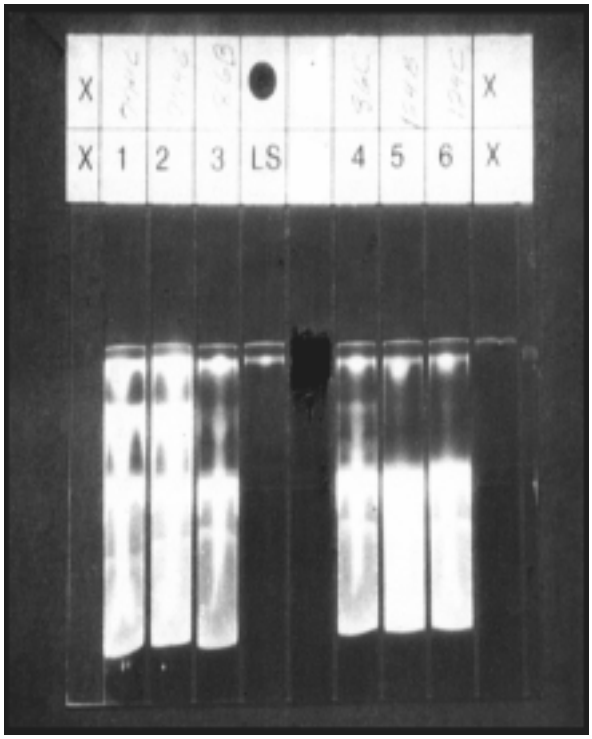
If the standard does not become visible the whole test has failed and will need to be repeated.

Note 17

Make sure the lamp is "off" if the plate is developed inside the viewing box. Do not look at the lamp directly. Do not leave the lamp resting in position when not in use (an earthquake may dislodge it, causing damage).

The development of colour for some sulphonamides may take longer than 15 minutes. If any potential presumptive positive samples are seen on the TLC plate, it is important to turn off the lamp and leave the plate in the dark for a further 15 minutes (total time after spraying is now 30 minutes) and view the plate again. The plate may be left for a further 15 minutes (45 in total) to confirm any suspicions, as the intensity of the bands may increase with time.

The most likely cause is the degradation of the fluorescamine in acetone solution and this can be checked by testing just a standard again. If no colour is seen, check the expiry date and request more fluorescamine from the laboratory.



Step 18

Looking across the plate, observe the sulphamethazine standard and note if any similar colour is in any of the other sample lanes at the same height. Mark the upper and lower bounds with pencil. Note if there is any similar greenish-yellow fluorescence colour in the zone within 1 cm of the solvent line (2 cm mark), as a sulphonamide other than sulphamethazine may be present.

Mark the upper and lower bounds of the standard band and any presumptive positive samples with a pencil on the TLC plate.

Record the distance the solvent travelled from the top of the loading zone and also the distance of the presumptive positive band above the loading zone. Provide this information to the laboratory when submitting confirmatory samples.

Note 18

The bands of colour **must be** greenish-yellow and are usually 2-3 mm high. They may be cusp shaped, curved or more intense at the margins or even divided, whereas the standard is almost always a solid band. Any of these variations in appearance from the standard, if present, could still indicate a presumptive positive sulphonamide.

When sending confirmatory samples to the laboratory, indicate whether the intensity of the fluorescence from the presumptive positive SOS test sample is more or less than the sulphamethazine standard.

7.3 Positive samples

Step 19

Any colour as per Step 18 is considered as indicating a **suspected positive** sample.

Note 19

If possible, have your observations checked by another person competent to perform the test. Record all results and observations on the form (see Appendix I of this manual — SOS Results) including details of the appearance of the plate and any measurements. Sign and date the results and have the person who checked the plate do likewise. Collate and file the forms.

Step 20

Tissues from bobby calves which have returned a suspected positive in this test will need to be analysed by a specialist laboratory to confirm the presence of sulphonamide drugs. Follow the relevant MAF Food specifications.

Note 20

Ensure the samples are taken according to the MAF Food specifications.

8. Ordering of Supplies

8.1 Prior to the start of the bobby calf season, the Technical Supervisor shall ensure that sufficient supplies of replacement items are available and have not exceeded any expiry date.

8.2 An inventory check should be made to identify any shortfall, based on expected throughput and sampling requirements. If there is a shortfall in replacement supplies, an order stating the quantity of each replacement item required shall be made using Appendix II, and forwarded by fax to the SOS Programme Co-ordinator for supply.

8.3 The orders for replacement items must be received at least 2 weeks in advance of the start of the bobby calf season and SOS testing.

8.4 Sulphonamide standards and fluorescamine dry powder will be automatically supplied on monthly basis. If either the fluorescamine or the standards are used up within this 1-month period, premises will need to reorder as necessary.

8.5 A check of equipment (listed as durables on Page 3.1) should also be undertaken to ensure the kit is operational.

Appendix I. SOS Results

Date: _____

Analyst: _____

Checked: _____

Appendix II. SOS Order for Supplies

SOS Order for Supplies

Please complete this form and fax it to the SOS Programme Co-ordinator, NCRL
Fax: (04) 528 1375

Premises Name: **Licence No:**

Date: _____

Ordered by: _____

Item	Quantity to be Ordered	Quantity Sent
Sulphamethazine standard		
Fluorescamine powder		
TLC plates		
Wiretrol capillaries		
Transfer pipettes		
Centrifuge tubes		
Vacutainers		
Vacutainer needles		
Methanol (2.5 l)		
Ethyl acetate (2.5 l)		
Acetone (2.5 l)		
For Laboratory Use		
Date Sent	Courier	
Initials		

Please send the account for supplies to:

Name: _____
Address: _____
_____