



# Product Protocol

<b>Product Name</b>	<b>Jones' PAS-M Stain Kit</b>
---------------------	-------------------------------

<b>Product Code</b>	<b>RRSK303-100</b>
---------------------	--------------------

<b>Reagents</b>	
Periodic Acid 1% aqueous	1x 50ml
Hexamine (Methanamine) 3% solution	4x 250ml
Silver Nitrate 5% solution	1x 50ml
Sodium Tetraborate 5% solution	2x 50ml
Gold Chloride 0.1% solution	1x 50ml
Sodium Thiosulphate 2% solution	1x 50ml
Neutral Red 0.1% aqueous solution	1x 50ml

<b>General Information</b>	
Number of Tests:	100 (based on coplin jar staining in batches of 5)
Procedure Time:	105 minutes (post warming of working solution) (approximate)
Shelf Life:	3 Years (from date of manufacture)
Storage:	Silver Nitrate 2-8C. Other reagents 15-25 °C

**Principle**

The Jones' silver methenamine staining technique is used to demonstrate basement membrane of the glomerulus in the kidney. The carbohydrate components are first oxidised with periodic acid solution to produce aldehydes which subsequently reduce silver to a visible metallic silver. Excess silver is removed by treatment with sodium thiosulphate

**Specimen Collection**

Tissue should be fixed in Formaldehyde or Bouins fixative for best results. Tissue should then be processed and embedded in paraffin wax. Sections should be cut at about 3 microns

**Working Solution**

Hexamine-Silver Working solution:  
 In a chemically clean container measure 50ml of hexamine and 2.5ml of 5% silver nitrate. A white precipitate forms which will dissolve on shaking. Add 5ml of 5% sodium tetraborate. Make fresh every time  
 Filter the solution into a Coplin jar and put into an oven or water bath at 56°C in darkness. Check temperature of solution before starting

- Protocol**
1. Dewax sections, hydrate through alcohols and rinse in tap water
  2. Oxidise the sections with periodic acid for 20-30 minutes at room temperature
  3. Rinse in distilled water
  4. Stain sections in the pre-heated working silver solution at 56°C (see note 1) for 20 mins initially (Check microscopically after washing in distilled water – basement membranes should be well demonstrated). If staining not complete return to the heated silver solution for 5 minute intervals and repeat the examination process
  5. Wash in several changes of distilled water



## Product Protocol

### Protocol (Contd)

6. Tone sections with gold chloride for 2 minutes
7. Wash in several changes of distilled water
8. Treat with sodium thiosulphate for 2 minutes
9. Wash in distilled water and counterstain in neutral red solution for 30 seconds
10. Rinse in water, dehydrate, clear and mount

### Results

Basement Membrane	Black
Background	Pink/Red

### Notes

1. Samples which have been treated in a Coplin jar can be incubated in a microwave at 500W if preferred for 1 minute.
2. All glassware/plasticware used in this procedure must be chemically clean and well washed in distilled water. Avoid the use of metal forceps with silver stains.. Use plastic forceps or plastic coated metal forceps to handle slides.
3. Always use a known control containing i.e. fungi, basement membrane
4. Component bottle Sizes have been supplied which are as close as possible to the number of tests needed. There may be occasion where more product than necessary is supplied, please either discard or use as require
5. Staining times may be modified to provide varying intensities of staining.

### Stability

If correctly stored the reagents are usable until the expiry date

### Disposal

Hazardous Reagents Included, observe local waste disposal regulations