6.0 APPENDICES

A: ETHICS CLEARANCE CERTIFICATE

B: SUBJECT INFORMATION & CONSENT FORM

C: CELL BLOCK PREPARATION

D: PAP STAIN

E: DIFF-QUICK STAIN

F: H&E STAIN

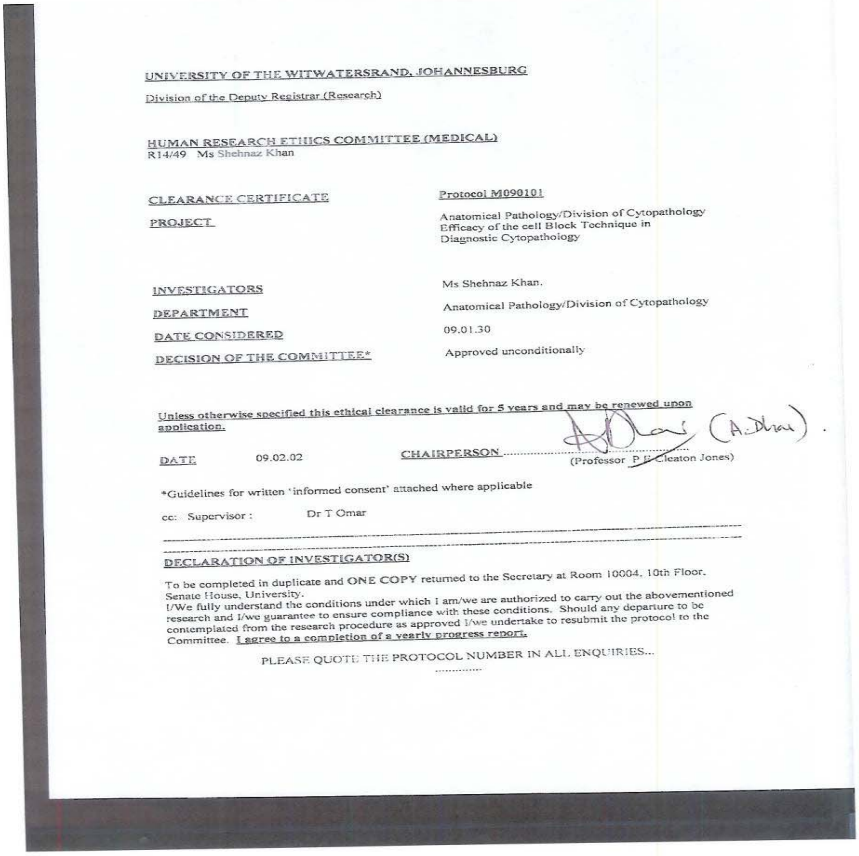
G: IMMUNOCYTOCHEMISTRY STAIN

H: DESTAINING PAP-STAINED SLIDES

I: STATISTICAL DATA

APPENDIX A:

ETHICS CLEARANCE CERTIFICATE



UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Ms Shehnaz Khan

CLEARANCE CERTIFICATE	Protocol M090101
PROJECT	Anatomical Pathology/Division of Cytopathology Efficacy of the cell Block Technique in Diagnostic Cytopathology
INVESTIGATORS	Ms Shehnaz Khan.
DEPARTMENT	Anatomical Pathology/Division of Cytopathology
DATE CONSIDERED	09.01.30
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 09.02.02 CHAIRPERSON ..... (A.Khan)
(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Dr T Omar

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and ONE COPY returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. I agree to a completion of a yearly progress report.

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

APPENDIX B:
SUBJECT INFORMATION & CONSENT FORM

SUBJECT INFORMATION FOR PARTICIPANTS
AND
CONSENT FORM

Study Title: "EFFICACY OF THE CELL BLOCK TECHNIQUE IN DIAGNOSTIC CYTOPATHOLOGY".

Hello, my name is Shehnaz Khan and I am from the Department of Cytopathology of the National Health Laboratory Services, and the University of Witwatersrand. We are inviting you to participate in this research study that looks at different laboratory tests in order to improve the diagnosis and treatment of various diseases. Your participation in this study is voluntary. No one can force you to participate if you don't want to.

- **Why are we doing this study?**

We are doing this study because we would like to develop a test to improve the diagnosis and treatment of various diseases.

- **What will happen during this study / What will happen if I agree to join the study?**

Before your FNA procedure, trained staff will talk to you to make sure you understand what the study is all about. You will also be given a chance to ask questions about the study or this form.

As your doctor has already explained to you: A fine needle aspiration procedure (FNA) is required in order to have your lump (specimen) examined using a microscope. During this procedure more material (less than a quarter teaspoon) will be obtained from your lump. This will require an extra prick with the tiny needle which is used for the FNA procedure. This will not interfere with the laboratory examination of your specimen in any way. The extra material will be used to test new methods for diagnosis.
Any spare material will be stored at the Division of Cytopathology according to routine storage procedure.

If you wish to join the study, please sign your name at the end of this form.

Your signature means that you have read this and understood the information given to you about this study. You will NOT be giving away your rights by signing this consent form and you can withdraw your consent at any time.

- **What will happen if I do not want to join the study?**

If you do not want to be part of this study, you will continue to receive the same treatment as any of the other patients here. The doctors treating you will not know whether or not you've agreed to this study and will not discriminate against you for not joining this study.

- **What are the benefits of participating in the study?**

There may be no immediate benefits to you in joining the study and you will not be paid. Whilst this test is not in routine use yet, the results of the test may or may not assist in the diagnosis of your condition. However your participation in this study will help us to develop tests to improve the diagnosis and treatment of various diseases in the future.

- **What are the risks and discomforts involved?**

An extra prick with the tiny needle used for the FNA procedure is required to obtain your sample.

- **Will my information be kept private?**

Yes, all study information will be kept private. The results will be made known to you (if you want) by your doctor and we will use the information we found in the study to write research reports and research papers. Throughout the study, information that could identify you will be kept confidential. Only people involved with the study will see your information.

- **If I change my mind later, may I withdraw from the study?**

Yes, you are joining at your own free will. You may withdraw from the study at any time. Just let me know. You will still be treated properly here.

- **Contact details of the researchers?**

Mrs Shehnaz Khan (Principal Investigator) 073 334 3668
Dr T Omar (Supervisor)

CONSENT FORM

If you want to be part of this study, please sign below.

_____ _____ _____

Name of Participant (Printed) Date Signature / mark / thumbprint

Person conducting informed consent:

_____ _____

Name (Printed) Date

Signature

Witness who was present for entire consenting procedure (for participants who cannot read and write):

_____ _____

_____

Name of Witness Date

Signature

I thank you for your participation, as this study would not be possible without your contribution.

APPENDIX C:

CELL BLOCK PREPARATION

REFERENCES: Shandon Cytoblock – cell preparation system – package insert.
Shandon Formal-Fixx – package insert.

EQUIPMENT: Cytospin, Shandon cellblock kit.

PRINCIPLE:
After a fine needle aspirate has been taken, the needle may be rinsed in a suitable fixative or a dedicated needle aspiration is obtined. This suspension of cells may be prepared in such a way as to render it similar to a paraffin embedded histology block. The advantage of this technique is that multiple sections may be taken allowing multiple procedures such as immunocytochemistry or special stains to be performed on a relatively scanty amount of material. This method makes use of the Shandon Cytospin machine and the Cytoblock kit.

FIXATIVE:
The specimen is collected in the collection fluid (Shandon Formal-Fixx). The cell button that is formed during the cell block preparation is fixed in Shandon Formal Fixx solution (which is unbuffered). Do not use phosphate-buffered solutions during any processing step as this interferes with the cell matrix which is formed.

SOLUTION:
All solutions required to make a cell block are included in the Cytoblock Kit.

PROTOCOL:

1. The needle-rinse specimen or dedicated needle aspiaration is collected in Formal-Fixx. Dilute the concentrate as follows:
   1 part Formal-Fixx concentrate (500 ml)
   4 parts de-ionized water (2000ml)

2. Continue with method as described in Cytoblock Cell Block Preparation System instruction manual (from page 1), which is as follows:

3. Centrifuge cell suspension for 1 minute at 25 000 rpm.
4. Estimate amount of specimen present. Each block should have 2 drops of specimen or less. Add 4 drops of Reagent 2 to 2 drops or less of specimen pellet and mix by vortexing gently.
5. Assemble Cytoblock cassette into Cytoclip.
6. Apply 3 drops of Reagent 1 onto centre of well in the board insert.
7. Place assembled Cytoclip into the Cytospin.
8. Place the mixed cell suspension in each Cytofunnel.

9. Close the Cytospin and spin for 5 minutes at 1500 rpm, at low acceleration setting. Remove Cytofunnel assemblies carefully.

10. Place 1 drop of Reagent 1 in the centre of the insert board well, on top of the cell button. Close the Cytoblock cassette and place in fixative to await processing.

11. Cell Block Processing schedule:

BOTTLE	SOLUTION	TEMP	TIME
1	Formal-Fixx	45^0C	20 min
2	Formal-Fixx	45^0C	5 min
3	80% Alcohol	45^0C	5 min
4	95% Alcohol	45^0C	5 min
5	95% Alcohol	45^0C	5 min
6	Absolute Alcohol	45^0C	5 min
7	Absolute Alcohol	45^0C	5 min
8	Absolute Alcohol	45^0C	5 min
9	Xylene	45^0C	5 min
10	Xylene	45^0C	5 min
11	Wax	60^0C	5 min
12	Wax	60^0C	5 min
13	Wax	60^0C	5 min
14	Wax	60^0C	5 min

4. Embedding:

a) The cassettes are removed from the wax bath and embedded in paraffin wax using a stainless steel tissue embedding mould of relevant size (small, medium or large)

b) Embed one block at a time to prevent specimens from being mixed.

c) Dispense wax into the selected mould. Holding the tissue with forceps, quickly place it into wax (before the wax hardens). The tissue may be orientated (if required), during this stage. Place the labelled cassette into mould. Fill with wax and place onto cold plate to harden.

d) It is easily removed from the metal mould.

e) The temperature of the paraffin wax used in the tissue embedder and tissue processor is between 55-60^0C.

5. Tissue sectioning (Microtome):

The clot, embedded in wax, is then sectioned on a microtome at 3-4 microns as follows:

(1) Fix the block in the block-holder on a microtome in such a position that it will be clear of the blade when in this position.

(ii) Turn back the feed mechanism on the microtome almost as far as it will go.

(iii) Insert a new disposable microtome blade and secure in position; check that the tilt of the blade holder is set at the correct angle (5^0).

(iv) Adjust the feed mechanism until the wax block is almost touching the blade.

(v) To trim the block set the section thickness gauge to about 15 microns and operate the microtome until complete sections of the clot are being cut. Alternatively, blocks may be trimmed manually.

(vi) Move the blade to new position. Apply ice to the surface of the block for a few seconds, and wipe the surface of the block free of water. Alternatively, place blocks directly onto ice tray.

(vii) Set thickness gauge to 3 microns and operate the microtome until complete sections are being cut.

(viii) Sections are floated onto the surface of water, in a water bath, where the temperature of the water does not exceed 45-50ºC. The sections are then picked up with glass slides, allowed to first air dry and then dry for about 30 minutes at 60ºC.

(xi) Before cutting the next block, the surface of the waterbath is skimmed (using tissue paper) to remove tissue residue of previous block.

(x) The water in the waterbath is changed at least once a day.

6. Haematoxylin and Eosin staining:

The slides are then stained with haematoxylin and eosin. (See Haematoxylin and Eosin Method, Appendix F).

APPENDIX D:

PAPANICOLAOU STAIN

REFERENCE: Comprehensive Cytopathology, Marluce Bibbo MD

1. Remove slides from 95% alcohol.
2. Immerse in 70% alcohol for 30 seconds.
3. Rinse slides in running water for 30 seconds.
4. Stain in Gills Haematoxylin for 4 minutes.
5. Rinse slides in running water for 1 minute.
6. Immerse in Scott's tap water for 1 minute.
7. Rinse slides in running water for 1 minute (2 changes).
8. Immerse in 70% alcohol for 30 seconds.
9. Immerse in 80% alcohol for 30 seconds.
10. Immerse in 95% alcohol for 30 seconds.
11. Stain in OG-6 II for 2 minutes.
12. Immerse in 95% alcohol for 30 seconds (2 changes).
13. Stain in EA 65 for 3 minutes.
14. Immerse in 95% alcohol for 30 seconds (2 changes).
15. Immerse in absolute alcohol for 30 seconds (3 changes).
16. Immerse in 50%xylene and 50% alcohol mixture for 30 seconds.
17. Immerse in xylene for 30 seconds.
18. Immerse in xylene for 5 minute.
19. Coverslip slides.

RESULTS:

Nuclei	-	blue/black
Cytoplasm (non-keratinising squamous cells)	-	blue/green
Keratinising cells	-	pink/orange

APPENDIX E:

DIFF-QUICK STAIN

REFERENCE: Comprehensive Cytopathology, Marluce Bibbo MD

1. Immerse slides in methanol for 10 minutes.
2. Stain in Haematoxylin by immersing slides in solution for 30 dips.
3. Stain in Eosin by immersing slides in solution for 30 dips.
4. Stain in Methylene Blue by immersing slides in solution for 30 dips.
5. Rinse in distilled water.
6. Allow to dry.
7. Clear in xylene for 5 minutes
8. Coverslip slides.

RESULTS:

helicobacter - dark blue

background - light blue

platelets - violet to purple

neutrophils: nucleus - dark blue
cytoplasm - pale pink

eosinophils: nucleus - blue
cytoplasm - blue
granules - red to red/orange

basophils: nucleus - purple or dark blue
granules - dark purple/black

monocytes: nucleus(lobated) - violet
cytoplasm - sky blue

APPENDIX F:

HAEMATOXYLIN & EOSIN (H&E) STAIN

METHOD:

1. Dewax sections in xylene and hydrate through graded alcohols. Rinse well in water.

2. Place in Mayer's Haematoxylin for 7 minutes. Wash well in running tap water and allow sections to blue in tap water.

3. Drain off excess water.

4. Place in Scott's tap water for 1-2 minutes and allow sections to blue. Wash well in running tap water.

5. Place in Eosin Solution for 1-2 minutes. Wash quickly to remove excess stain and drain slides on paper towel. (It is preferable to air dry slides or place in incubator at 37ºC for about 20 minutes before proceeding with the next step).

6. Dehydrate, clear and mount the slides.

RESULTS:

Nuclei	-	blue
Cytoplasm	-	pink
Erythrocytes	-	orange
Background material	-	pink

APPENDIX G:

IMMUNOCYTOCHEMISTRY TEST ON CYTOLOGICAL AND CELL BLOCK MATERIAL

PURPOSE: To aid Pathologists in making an accurate diagnosis, using a selective panel of antibodies.

REFERENCES: Theory & Practice of logical Histological Techniques, JD Bancroft.

EQUIPMENT:

Pressure Cooker
pH Meter
Adjustable volume Pipettes
Perspex Humidity Tray

PRINCIPLE:
Immunocytochemistry is the technique whereby an antigen reacts with an antibody specific to the antigen. The antibody must be labelled with a suitable marker that will allow identification. Visualization of this reaction is achieved by labelling the antibodies with an enzyme such as horse radish peroxidase followed by a diaminobenzidine substrate. A dark-brown permanent stain is obtained which is easily recognised microscopically.

FIXATIVE:
Alcohol-based fixative is ideal for immunocytochemistry. Good results are obtained using Cytological Spray Fixative (Fencott) on both unstained and previously Papanicolaou-stained slides.

CONTROLS:
Lack of available cytological material has made tissue controls a suitable alternative provided the necessary steps are taken to expose the antigen, namely by enzyme digestion or heat induced epitope retrieval. Positive and negative controls are essential - a positive control to check that the technique is working optimally and a negative control to exclude false positive results.

Positive Control: Respective paraffin wax embedded tissue control as indicated in product specification sheet.

Negative Control: A representative portion of the test slide is used. The incubation step with primary antibody is omitted – antibody diluent is applied instead.

SMEAR SLIDES:
Smear slides may be carefully cut with a glass cutting knife (tungsten carbide) into segments (depending on number of tests requested) to allow for more than one immunochemical test and a negative control. It is usually possible to obtain about 3-4 segments per slide. These are then re-assembled at the end of the procedure.

ANTIGEN RETRIEVAL METHODS:

The following methods are used only on formalin-fixed tissue sections. These include control tissue, clots and cell blocks. These techniques do not apply to smears and in most cases will destroy the material.

i) Enzymatic Digestion:

Trypsin Digestion: (Stored at 4º C)

1. De-wax and hydrate tissue section in running tap water. Thereafter rinse in distilled water.

2. Apply 2 drops of Trypsin (concentrate) Digest-All 2A and add 2 drops of Trypsin diluent – Digest-All 2 B.

3. Cover gently with a coverslip making sure that there are no air bubbles trapped between coverslip and slide.

4. Incubate for at 37ºC for 15 minutes.

5. Wash in several changes of running tap water and thereafter distilled water.

6. Resume immuno staining procedure from the methanol/hydrogen peroxide step.

Pepsin Digestion: (Digest-All Pepsin solution from ZYMED stored at 4ºC)

1. De-wax and hydrate tissue section in running tap water. Thereafter rinse in distilled water.

2. Apply 2-3 drops of Pepsin to section. Cover gently with a coverslip making sure that there are no air bubbles trapped between the slide and the coverslip.

3. Incubate at 37ºC for 5 minutes.

4. Wash in several changes of running tap water and thereafter distilled water.

5. Resume immunostaining procedure from the methanol/hydrogen peroxide step.

Proteinase K: (Stored at 4º C, DakoCytomation)

6. De-wax and hydrate tissue section in running tap water. Thereafter rinse in distilled water.

7. Apply 2 drops of Pepsin.

8. Cover gently with a coverslip making sure that there are no air bubbles trapped between coverslip and slide.

9. Incubate for at room temperature for 5 minutes.

10. Wash in several changes of running tap water and thereafter distilled water.

11. Resume immuno staining procedure from the methanol/hydrogen peroxide step.

ii) HIGH TEMPERATURE UNMASKING TECHNIQUE (PRESSURE COOKER)

(as an alternative to the pressure cooker, the microwave may be used instead at medium power for 5 minutes, followed by a 5 minute cooling in the microwave and then for another 5 minutes at medium power).

A. 0.01M Citrate Buffer pH 6.00

(i) 0.1M Citric Acid Stock Solution (Stock A):

Citric Acid	21.01g
Distilled Water	1000ml

(ii) 0.1M Tri-sodium Citrate Stock Solution (Stock B):

Tri-sodium citrate	29.41g
Distilled Water	1000ml

Store stock solutions at 4ºC. Working citrate buffer solution should be made just before use.

Make up citrate buffer as follows:

a. Citric acid stock solution 9ml
b. Tri-sodium citrate stock solution 41ml
c. Distilled water 450ml

Adjust the pH of the buffer to pH 6.00 using the tri-sodium citrate (to increase pH) and / or citric acid (to decrease pH).

B. Citrate Buffer pH 6.10

Prepare as for citrate buffer pH 6.00 (above). However the pH is adjusted as follows:

Adjust the pH of the buffer to pH 6.10 using a 1M Sodium Hydroxide (to increase pH) and / or 1M HCL (to decrease pH).

C. Tris-Edta Buffer pH 9.00

Tris (base)	0.605g
EDTA	0.185g
Distilled Water	500 ml

Adjust pH to 9.00 with 1M NaOH (↑pH) and / or 1M HCL to (↓ pH).

D. EDTA Buffer pH 8.00 (Perform Retrieval in **Microwave** as indicated on page 6)

EDTA	0.185g
Distilled water	500 ml

Adjust pH to 8.00 with 1M NaOH (↑pH) and / or 1M HCL to (↓ pH).

E. 1M NaOH

NaOH	20g
Distilled water	500ml

F. 1M HCL

HCL	48.28ml
Distilled water	500 ml

Procedure for Heat Induced Epitope Retrieval (HIER):
1. De-wax and hydrate tissue section in running tap water. Thereafter rinse in distilled water.
2. Place slides in the respective rack and fill the container (of the pressure cooker) with the respective buffer.

3. Any empty spaces in the rack must be filled with blank slides. This ensures an even heat distribution.
4. Only pressure cook TWO containers at a time.
5. Place 500ml distilled water into the pressure cooker.
6. Load the containers (with the lid on) into the pressure cooker.
7. Place a Pascal quality strip on the top of one of containers
8. Seal on the lid securely.
9. Set pressure cooker to 110ºC for 30 seconds.
10. Press start.
11. When the procedure is complete a beeping sound is heard. Immediately record the pressure and temperature reading on the Pascal Pressure Cooker-Quality Control log.
12. Do not open the pressure cooker before the pressure gauge reaches zero.
13. As an extra precautionary step: Before opening the lid, lift the pressure release valve.
14. Remove containers and place on bench to allow slides to cool in the buffer for 30- 45 minutes.
15. Rinse slides in 2-3 changes of distilled water.
16. Resume immunostaining from methanol -hydrogen peroxide step.

Alternative (Contingency Plan for HIER): Microwave:

De-wax and hydrate tissue section in running tap water. Thereafter rinse in distilled water.

Place slides to be microwaved in a glass rack. Place rack in a 2000ml plastic beaker. Pour in the respective buffer and microwave on medium power for 5 minutes to warm buffer. Thereafter place slides into warmed solution and microwave at medium power for 15 minutes (EDTA) and 10 minutes on high for citrate buffer. Remove beaker from microwave oven and allow slides to cool in the buffer at room temperature for 20-30 minutes.

Rinse well in distilled water.

Resume immunostaining from methanol -hydrogen peroxide step.

REAGENT PREPARATION:

A. WASH BUFFER

Wash buffer concentrate	200ml
Distilled water	1800ml

B. 6% HYDROGEN PEROXIDE IN COLD METHANOL

Hydrogen peroxide (30 vol / 100%)	6ml
Cold Methanol	100ml

C. Alternative (Contingency Plan): for Wash Buffer

(i) Tris – HCL pH 7.6

Tris –HCL	15.76g
Tris – Base	12.12g
Sodium Chloride	17.54g
Tween 20	1 ml
Distilled Water	2000 ml

Dissolve in 2 litres distilled water. pH to 7.6 with 1M NaOH (↑pH) and / or 1M HCL to (↓ pH). Add 1 ml Tween 20 and allow to mix until dissolved.

(ii) TBS sachets (DakoCytomation) pH 7.6

Dako TBS sachet	x1
Distilled water	1000 ml
Tween 20	0.5 ml

Dissolve ONE TBS sachet in 1000 ml distilled water. No need to pH. Add 0.5 ml Tween 20 and allow to mix until dissolved.

<u>METHOD FOR ICC:</u>

1. Remove coverslips from pre-stained slides by soaking in xylene (glass coverslips) or acetone (coverslip film) and hydrate through graded alcohols to running tap water.

2. Decolourise test slides in 1% acid alcohol (1% Hydrochloric acid in 70% alcohol) for 2-3 minutes. Rinse well in running tap water for 10 minutes. Thereafter rinse slides in distilled water. [Tissue controls and clot slides that have been formalin-fixed and paraffin embedded tissue do not require to decolourisation].

3. De-wax and hydrate tissue controls and / or clot slides. Perform antigen retrieval methods (as discussed earlier) for those tests that require pre-treatment e.g. enzymatic or high temperature unmasking, etc. Once completed place in distilled water.

4. Place slides in 6% hydrogen peroxide in cold methanol for 30 minutes.

5. Rinse slides in 2-3 changes of distilled water.

6. Correlate the slide's Reference number to its corresponding immuno request form. Place the slide with the smear facing upwards onto the schematic diagram of a slide on the request form. Using a glass-cutting

knife with a tungsten –carbide blade, cut the slide accordingly or divide the slide appropriately into the number of tests required. Always leave a section for a negative control. Etch (with a diamond pen), the temporary case number and name of the antibody to be used. (This process may be performed after the hydrogen peroxide – methanol step).

7. Rinse slides / pieces in 2-3 changes of wash buffer.

8. Incubate sections in respective primary antibody. Dilute in antibody diluent and incubate according to specifications. Most antisera require incubation of 1 hour at room temperature. Refer to specifications labelled onto each antibody. Apply 100 µl of primary antibody and cover all slides / pieces with a coverslip.

9. Rinse slides / pieces thoroughly in 2-3 changes of wash buffer.

10. Apply 2 drops of Envision Detection System per slide / piece and incubate at room temperature for 30 minutes.

11. Some antibodies require a separate detection system. Refer to specifications as indicated above.

12. Rinse slides / pieces thoroughly in 2-3 changes of wash buffer.

13. Using gloves and a mask, make up a Diaminobenzidene (DAB) solution (supplied in Envision kit as a liquid) as follows:

Bottle B	1ml
Bottle A	20 µl

Use within 10 minutes of making. **NB.: See Hazard note**

14. Treat slides with DAB solution for 6-7 minutes. Discard DAB solution accordingly – see below-Neutralisation of DAB).

15. Place slides / pieces in coplin jars containing wash buffer or distilled water.

16. Rinse in tap water thoroughly to remove any residual DAB.

17. Stain nuclei with Mayer's haematoxylin (filter before use) for 10 seconds.

18. Rinse well in tap water.

20. Dehydrate, clear and mount. Re-assemble smear slides.

RESULTS:
Antigen-Antibody Reaction - brown

Nuclei - blue

EVALUATION: The results are evaluated by the pathologist.

HAZARDS AND NEUTRALISATION OF DAB:
Diaminobenzidine (DAB) is a potential carcinogen and appropriate safety precautions should be taken at all times.

1. When handling DAB wear gloves, a mask and an eye-shield.
2. Neutralise by adding 50ml 0.2 M potassium permanganate and 50 ml 2M sulphuric acid to every 100ml of DAB and allow to stand overnight.
3. Add ascorbic acid until solution turns colourless.
4. Add sodium bicarbonate until solution stops fizzing.
5. Optional: Check that the pH of solution is at 7.00 using pH indicator sticks.
6. Solution is now safe to discard down the drain.

Note: Although the amount of DAB used in the Immunocytochemistry procedure is much less than 50ml since it is in liquid form, the same volumes as stipulated above are used.

PREPARATION OF REAGENTS FOR DAB NEUTARLISATION:

0.2 M potassium permanganate

Dissolve 31.60g potassium permanganate in 1000ml distilled water.

2M Sulphuric Acid

Concentrated sulphuric acid	54.9 ml
Distilled water	1000 ml

CAUTION: Always add acid to water – *gradually*.

NOTES:

a. If immuno is to be performed on paraffin-embedded clotted material, cut a section for each test to be performed plus one extra slide to be used as a negative control. These sections should be floated onto adhesive coated slides or charged slides to prevent loss of material during the immunocytochemistry procedure. Allow sections to dry overnight between 37ºC- 40ºC.

COATING OF SLIDES FOR MICROWAVE TECHNIQUE (ALTERNATIVE FOR SUPERFROST SLIDES)

STA – ON SLIDES

MATERIALS:
STA-ON Tissue section Adhesive
Distilled water

Slides
Waterbath (tissue section floatation bath)

METHOD:

1. Dilute 20 ml STA-ON reagent in 1000ml distilled water already prewarmed (in waterbath) to 40°C – 46°C.

2, Immerse slides (already packed in a slide rack) in solution for 30 minutes.

3. Allow slides to air dry overnight at room temperature or at 37ºC.

4. Once dry, pack slides in clean slide boxes and store at 4 ºC for about 2-3 months.

APPENDIX H:

DESTAINING PAP-STAINED SLIDES

PURPOSE:
To ensure that the correct procedure is followed for the de-staining of smears.

REFERENCE: Comprehensive Cytopathology – Marluce Bibbo MD

PREPARATION OF ACID-ALCOHOL SOLUTION:

To make up 1000 ml use the following:

700 ml absolute alcohol
300 ml distilled water
10 ml concentrated HCI
Mix together

NOTE:

Always add acid to alcohol - because an explosive chemical reaction can occur if the process is reversed.

DESTAINING AND RESTAINING TECHNIQUE:

1. Immerse slide in xylene and allow to soak until coverslip falls off. The time needed is variable.
2. Check the slide at selected time intervals until the old mounting medium is also removed completely.
3. Rinse the slide to clear in clean xylene.
4. Rinse slide in Absolute alcohol.
5. Place in acid alcohol until slide is completely colourless.
6. Stop acid alcohol reaction by rinsing the slide in distilled water.
7. Re-stain using the requested staining techniques.
8. Stain and mount as usual.

NOTE:
Should a Diff quick stained slide need to be de-stained, the slide can be rinsed in Methanol after the coverslip and mounting medium have been removed. The time limit in the Methanol varies with the shade of stain required.

APPENDIX I:

STATISTICAL DATA

20 Oct 2010, 13:33:55

```
. symmetry   cell_h_p1 cell_h_p2

-------------------------------------------
1         |            2 cell_h_p
cell_h_p  |   0      1      2      3    Total
----------+--------------------------------
        0 |    0      0      0      0      0
        1 |    1      2      0      2      5
        2 |    3      7      5      2     17
        3 |    0     10     12      6     28
          |
    Total |    4     19     17     10     50
-------------------------------------------

                                                  chi2     df     Prob>chi2
-------------------------------------------------------------------------------
Symmetry (asymptotic)                          |    23.48      5         0.0003
Marginal homogeneity (Stuart-Maxwell) |    19.61      3         0.0002
-------------------------------------------------------------------------------

. kap   cell_h_p1 cell_h_p2

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
  26.00%      26.56%    -0.0076     0.0756      -0.10      0.5402

. symmetry   cell_h_p1 cell_h_p2 if  ep_r==2
-------------------------------------------
1         |            2 cell_h_p
cell_h_p  |   0      1      2      3    Total
----------+--------------------------------
        0 |    0      0      0      0      0
        1 |    0      2      0      2      4
        2 |    1      5      4      1     11
        3 |    0      3      9      3     15
          |
    Total |    1     10     13      6     30
-------------------------------------------

                                                  chi2     df      Prob>chi2
-------------------------------------------------------------------------------
Symmetry (asymptotic)                          |    12.60      4         0.0134
Marginal homogeneity (Stuart-Maxwell) |     7.48      3         0.0581
-------------------------------------------------------------------------------
. kap   cell_h_p1 cell_h_p2 if  ep_r == 2

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
  30.00%      30.33%    -0.0048     0.1104      -0.04      0.5173
```

```
. gen  morph_h_p1_01 = ( morph_h_p1 > 0)

. gen  morph_h_p2_01 = ( morph_h_p2 > 0)

. symmetry    morph_h_p1_01 morph_h_p2_01

-------------------------------
morph_h_p |    morph_h_p2_01
1_01      |   0      1    Total
----------+--------------------
        0 |    0      0      0
        1 |    6     44     50
          |
    Total |    6     44     50
-------------------------------
                                           chi2     df      Prob>chi2
-----------------------------------------------------------------------------
Symmetry (asymptotic)                  |      6.00      1         0.0143
Marginal homogeneity (Stuart-Maxwell) |      6.00      1         0.0143
-----------------------------------------------------------------------------
. kap   morph_h_p1_01 morph_h_p2_01

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
  88.00%      88.00%     0.0000          .         .           .

. symmetry    morph_h_p1_01 morph_h_p2_01 if  ep_r == 2

-------------------------------
morph_h_p |    morph_h_p2_01
1_01      |   0      1    Total
----------+--------------------
        0 |    0      0      0
        1 |    3     27     30
          |
    Total |    3     27     30
-------------------------------

                                           chi2     df      Prob>chi2
-----------------------------------------------------------------------------
-
Symmetry (asymptotic)                  |      3.00      1         0.0833
Marginal homogeneity (Stuart-Maxwell) |      3.00      1         0.0833
-----------------------------------------------------------------------------
-

. kap   morph_h_p1_01 morph_h_p2_01 if  ep_r == 2

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
  90.00%      90.00%     0.0000          .         .           .
```

```
->  symmetry arch_h_p1 arch_h_p2

------------------------------
1          |     2 arch_h_p
arch_h_p   |   0      1    Total
-----------+--------------------
         0 |    0      0      0
         1 |   28     22     50
           |
     Total |   28     22     50
------------------------------
                                                chi2     df      Prob>chi2
------------------------------------------------------------------------------
-
Symmetry (asymptotic)                     |     28.00      1         0.0000
Marginal homogeneity (Stuart-Maxwell) |     28.00      1         0.0000
------------------------------------------------------------------------------

->  kap arch_h_p1 arch_h_p2

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
  44.00%      44.00%     0.0000     0.0000         .           .

->  symmetry arch_h_p1 arch_h_p2 if ep_r == 2

------------------------------
1          |     2 arch_h_p
arch_h_p   |   0      1    Total
-----------+--------------------
         0 |    0      0      0
         1 |   13     17     30
           |
     Total |   13     17     30
------------------------------
                                                chi2     df      Prob>chi2
------------------------------------------------------------------------------
Symmetry (asymptotic)                     |     13.00      1         0.0003
Marginal homogeneity (Stuart-Maxwell) |     13.00      1         0.0003
------------------------------------------------------------------------------

->  kap arch_h_p1 arch_h_p2 if ep_r == 2

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
  56.67%      56.67%     0.0000     0.0000         .           .

. log close
       log:  C:\DATA_10\shenaz.log
  log type:  text
 closed on:   3 Feb 2010, 10:55:02
------------------------------------------------------------------------------
-------------------------------------------------
```

15 Mar 2010, 09:54:39

```
. for var  ck71 -  ae13_b1  hep11 - ttf1b1  neg_b1 \ var  ck72 -
ae13_b2  hep12 - ttf1b2  neg_b2: symmetry X Y \ kap X Y \ symmetry X Y
if  ep_r == 2 \ kap X Y if  ep_r == 2

->  symmetry ck71 ck72

-------------------------------------------------------------
           |                        2 ck7
    1 ck7  |   0      1      2      3      5      6    Total
-----------+-------------------------------------------------
         0 |    6      0      0      0      0      0      6
         1 |    1      2      0      0      0      0      3
         2 |    1      0      2      0      0      0      3
         3 |    1      2      0      0      1      0      4
         5 |    2      2      0      0      0      2      6
         6 |    4      2      4      0      6      6     22
           |
     Total |   15      8      6      0      7      8     44
-------------------------------------------------------------

                                             chi2     df      Prob>chi2
-------------------------------------------------------------------------
-
Symmetry (asymptotic)                     |    22.00      11        0.0244
Marginal homogeneity (Stuart-Maxwell)     |    19.92      5         0.0013
-------------------------------------------------------------------------
-

->  kap ck71 ck72

             Expected
Agreement    Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
  36.36%      18.08%     0.2232     0.0632       3.53      0.0002

->  symmetry ck71 ck72 if ep_r == 2

-------------------------------------------------------------
           |                        2 ck7
    1 ck7  |   0      1      2      3      5      6    Total
-----------+-------------------------------------------------
         0 |    5      0      0      0      0      0      5
         1 |    1      2      0      0      0      0      3
         2 |    0      0      2      0      0      0      2
         3 |    0      1      0      0      1      0      2
         5 |    2      1      0      0      0      1      4
         6 |    3      1      3      0      3      2     12
           |
     Total |   11      5      5      0      4      3     28
-------------------------------------------------------------

                                             chi2     df      Prob>chi2
-------------------------------------------------------------------------
-
Symmetry (asymptotic)                     |     14.00      9         0.1223
```

```
Marginal homogeneity (Stuart-Maxwell) |     12.96      5         0.0238
-------------------------------------------------------------------------
-

->  kap ck71 ck72 if ep_r == 2

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
  39.29%      16.84%     0.2699     0.0779       3.46      0.0003

->  symmetry ck7_b1 ck7_b2

-----------------------------------------
          |               2 ck7_b
  1 ck7_b |   0      1      2      3    Total
----------+------------------------------------
        0 |   16      1      0      0     17
        1 |   11      1      0      0     12
        2 |    8      1      0      0      9
        3 |    2      2      2      0      6
          |
    Total |   37      5      2      0     44
-----------------------------------------

                                     chi2     df      Prob>chi2
-------------------------------------------------------------------------
-
Symmetry (asymptotic)                |     23.33      6         0.0007
Marginal homogeneity (Stuart-Maxwell) |     21.61      3         0.0001
-------------------------------------------------------------------------
-

->  kap ck7_b1 ck7_b2

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
  38.64%      36.52%     0.0334     0.0694       0.48      0.3155

->  symmetry ck7_b1 ck7_b2 if ep_r == 2

-----------------------------------------
          |               2 ck7_b
  1 ck7_b |   0      1      2      3    Total
----------+------------------------------------
        0 |    8      1      0      0      9
        1 |    6      1      0      0      7
        2 |    7      0      0      0      7
        3 |    2      2      1      0      5
          |
    Total |   23      4      1      0     28
-----------------------------------------

                                     chi2     df      Prob>chi2
-------------------------------------------------------------------------
-
```

```
Symmetry (asymptotic)                 |     15.57      5         0.0082
Marginal homogeneity (Stuart-Maxwell) |     14.79      3         0.0020
-------------------------------------------------------------------------
-

->  kap ck7_b1 ck7_b2 if ep_r == 2

            Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
  32.14%      30.87%     0.0185     0.0800       0.23      0.4088

->  symmetry ck201 ck202

-------------------------------------------------
          |                  2 ck20
   1 ck20 |   0      1      2      5      6    Total
----------+--------------------------------------
        0 |   35      0      0      0      0     35
        1 |    3      0      0      0      0      3
        2 |    3      0      0      0      0      3
        5 |    0      0      1      0      1      2
        6 |    0      0      0      1      0      1
          |
    Total |   41      0      1      1      1     44
-------------------------------------------------

                                        chi2     df      Prob>chi2
-------------------------------------------------------------------------
-
Symmetry (asymptotic)                 |      7.00      4         0.1359
Marginal homogeneity (Stuart-Maxwell) |      7.00      4         0.1359
-------------------------------------------------------------------------
-

->  kap ck201 ck202

            Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
  79.55%      74.43%     0.2000     0.0787       2.54      0.0055

->  symmetry ck201 ck202 if ep_r == 2

-------------------------------------------------
          |                  2 ck20
   1 ck20 |   0      1      2      5      6    Total
----------+--------------------------------------
        0 |   20      0      0      0      0     20
        1 |    3      0      0      0      0      3
        2 |    2      0      0      0      0      2
        5 |    0      0      1      0      1      2
        6 |    0      0      0      1      0      1
          |
    Total |   25      0      1      1      1     28
-------------------------------------------------
```

```
                                          chi2     df      Prob>chi2
------------------------------------------------------------------------
-
Symmetry (asymptotic)                  |      6.00      4         0.1991
Marginal homogeneity (Stuart-Maxwell) |      6.00      4         0.1991
------------------------------------------------------------------------
-

->  kap ck201 ck202 if ep_r == 2

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
  71.43%      64.41%     0.1971     0.0971       2.03      0.0212

->  symmetry ck20_b1 ck20_b2

-------------------------------------------
          |              2 ck20_b
 1 ck20_b |   0      1      2      3    Total
----------+--------------------------------
        0 |   18      1      0      0     19
        1 |   14      1      0      0     15
        2 |    6      2      0      0      8
        3 |    2      0      0      0      2
          |
    Total |   40      4      0      0     44
-------------------------------------------

                                          chi2     df      Prob>chi2
------------------------------------------------------------------------
-
Symmetry (asymptotic)                  |     21.27      4         0.0003
Marginal homogeneity (Stuart-Maxwell) |     20.24      3         0.0002
------------------------------------------------------------------------
-

->  kap ck20_b1 ck20_b2

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
  43.18%      42.36%     0.0143     0.0657       0.22      0.4137

->  symmetry ck20_b1 ck20_b2 if ep_r == 2

-------------------------------------------
          |              2 ck20_b
 1 ck20_b |   0      1      2      3    Total
----------+--------------------------------
        0 |   11      0      0      0     11
        1 |    9      0      0      0      9
        2 |    5      2      0      0      7
        3 |    1      0      0      0      1
          |
    Total |   26      2      0      0     28
-------------------------------------------
```

```
                                                chi2     df      Prob>chi2
------------------------------------------------------------------------------
-
Symmetry (asymptotic)                    |     17.00      4         0.0019
Marginal homogeneity (Stuart-Maxwell) |     15.77      3         0.0013
------------------------------------------------------------------------------
-

->  kap ck20_b1 ck20_b2 if ep_r == 2

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
------------------------------------------------------------------
  39.29%      38.78%     0.0083     0.0669       0.12       0.4505

->  symmetry syn1 syn2

--------------------------------------------------
           |                   2 syn
    1 syn |   0      1      2      5      6    Total
----------+---------------------------------------
        0 |    4      0      0      0      0      4
        1 |    0      0      0      0      0      0
        2 |    0      1      1      0      0      2
        5 |    1      0      0      0      0      1
        6 |    0      0      1      0      2      3
          |
    Total |    5      1      2      0      2     10
--------------------------------------------------

                                                chi2     df      Prob>chi2
------------------------------------------------------------------------------
-
Symmetry (asymptotic)                    |      3.00      3         0.3916
Marginal homogeneity (Stuart-Maxwell) |      3.00      4         0.5578
------------------------------------------------------------------------------
-

->  kap syn1 syn2

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
------------------------------------------------------------------
  70.00%      30.00%     0.5714     0.1829       3.12       0.0009

->  symmetry syn1 syn2 if ep_r == 2

-------------------------------------------
           |                2 syn
    1 syn |   0      1      2      6    Total
----------+--------------------------------
        0 |   3      0      0      0      3
        1 |   0      0      0      0      0
        2 |   0      1      0      0      1
        6 |   0      0      1      2      3
          |
```

```
    Total |   3      1      1      2      7
----------------------------------------------

                                              chi2     df      Prob>chi2
------------------------------------------------------------------------------
-
Symmetry (asymptotic)                  |      2.00      2         0.3679
Marginal homogeneity (Stuart-Maxwell) |      2.00      3         0.5724
------------------------------------------------------------------------------
-

->  kap syn1 syn2 if ep_r == 2

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
------------------------------------------------------------------
  71.43%      32.65%     0.5758     0.2397       2.40      0.0082

->  symmetry syn_b1 syn_b2

--------------------------------------
          |          2 syn_b
  1 syn_b |   0      1      2    Total
----------+---------------------------
        0 |    4      0      0      4
        1 |    4      1      0      5
        2 |    1      0      0      1
          |
    Total |    9      1      0     10
--------------------------------------

                                              chi2     df      Prob>chi2
------------------------------------------------------------------------------
-
Symmetry (asymptotic)                  |      5.00      2         0.0821
Marginal homogeneity (Stuart-Maxwell) |      5.00      2         0.0821
------------------------------------------------------------------------------
-

->  kap syn_b1 syn_b2

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
------------------------------------------------------------------
  50.00%      41.00%     0.1525     0.1517       1.01      0.1573

->  symmetry syn_b1 syn_b2 if ep_r == 2

--------------------------------------
          |          2 syn_b
  1 syn_b |   0      1      2    Total
----------+---------------------------
        0 |   3      0      0      3
        1 |   3      0      0      3
        2 |   1      0      0      1
          |
    Total |   7      0      0      7
--------------------------------------
```

```
                                                  chi2     df      Prob>chi2
-------------------------------------------------------------------------
Symmetry (asymptotic)                          |      4.00      2         0.1353
Marginal homogeneity (Stuart-Maxwell)          |      4.00      2         0.1353
-------------------------------------------------------------------------

->  kap syn_b1 syn_b2 if ep_r == 2

            Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
  42.86%      42.86%     0.0000          .          .           .

->  symmetry ae131 ae132

-------------------------------------------------------------
          |                       2 ae13
   1 ae13 |   0      1      2      3      5      6    Total
----------+--------------------------------------------------
        0 |   0      0      0      0      0      0      0
        1 |   0      0      0      0      0      0      0
        2 |   0      0      0      0      0      0      0
        3 |   0      0      0      0      0      1      1
        5 |   0      0      0      0      0      0      0
        6 |   1      1      1      0      2      1      6
          |
    Total |   1      1      1      0      2      2      7
-------------------------------------------------------------

                                                  chi2     df      Prob>chi2
-------------------------------------------------------------------------
Symmetry (asymptotic)                          |      6.00      5         0.3062
Marginal homogeneity (Stuart-Maxwell)          |      6.00      5         0.3062
-------------------------------------------------------------------------

->  kap ae131 ae132

            Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
  14.29%      24.49%    -0.1351     0.0791      -1.71      0.9562

->  symmetry ae131 ae132 if ep_r == 2

-----------------------------------------------------
          |                    2 ae13
   1 ae13 |   0      2      3      5      6    Total
----------+------------------------------------------
        0 |   0      0      0      0      0      0
        2 |   0      0      0      0      0      0
        3 |   0      0      0      0      1      1
        5 |   0      0      0      0      0      0
        6 |   1      1      0      2      0      4
          |
    Total |   1      1      0      2      1      5
-----------------------------------------------------
```

```
                                                chi2     df      Prob>chi2
------------------------------------------------------------------------
-
Symmetry (asymptotic)                    |      5.00      4         0.2873
Marginal homogeneity (Stuart-Maxwell) |      5.00      4         0.2873
------------------------------------------------------------------------
-

->  kap ae131 ae132 if ep_r == 2

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
   0.00%      16.00%    -0.1905     0.0852      -2.24       0.9873




->  symmetry ae13_b1 ae13_b2

-------------------------------------------
          |              2 ae13_b
 1 ae13_b |   0      1      2      3    Total
----------+--------------------------------
        0 |   0      0      0      0      0
        1 |   1      0      0      0      1
        2 |   3      1      1      0      5
        3 |   1      0      0      0      1
          |
    Total |   5      1      1      0      7
-------------------------------------------

                                                chi2     df      Prob>chi2
------------------------------------------------------------------------
-
Symmetry (asymptotic)                    |      6.00      4         0.1991
Marginal homogeneity (Stuart-Maxwell) |      5.57      3         0.1344
------------------------------------------------------------------------
-

->  kap ae13_b1 ae13_b2

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
  14.29%      12.24%     0.0233     0.0905       0.26      0.3986

->  symmetry ae13_b1 ae13_b2 if ep_r == 2

-------------------------------------------
          |              2 ae13_b
 1 ae13_b |   0      1      2      3    Total
----------+--------------------------------
        0 |   0      0      0      0      0
```

```
        1 |   1      0      0      0      1
        2 |   3      0      0      0      3
        3 |   1      0      0      0      1
          |
    Total |   5      0      0      0      5
----------------------------------------------
```

	chi2	df	Prob>chi2
Symmetry (asymptotic)	5.00	3	0.1718
Marginal homogeneity (Stuart-Maxwell)	5.00	3	0.1718

```
-> kap ae13_b1 ae13_b2 if ep_r == 2
```

Agreement	Expected Agreement	Kappa	Std. Err.	Z	Prob>Z
0.00%	0.00%	0.0000	0.0000	.	.

```
-> symmetry hep11 hep12

----------------------------------------------------
          |                  2 hep1
   1 hep1 |   0      1      2      5      6    Total
----------+-----------------------------------------
        0 |   6      0      0      0      1      7
        1 |   1      0      0      0      0      1
        2 |   1      0      0      0      0      1
        5 |   0      0      0      0      1      1
        6 |   0      0      0      0      0      0
          |
    Total |   8      0      0      0      2     10
----------------------------------------------------
```

	chi2	df	Prob>chi2
Symmetry (asymptotic)	4.00	4	0.4060
Marginal homogeneity (Stuart-Maxwell)	4.00	4	0.4060

```
-> kap hep11 hep12
```

Agreement	Expected Agreement	Kappa	Std. Err.	Z	Prob>Z
60.00%	56.00%	0.0909	0.1317	0.69	0.2451

```
-> symmetry hep11 hep12 if ep_r == 2
```

```
----------------------------------------------------
           |                    2 hep1
   1 hep1 |   0      1      2      5      6    Total
----------+-----------------------------------------
        0 |   5      0      0      0      0      5
        1 |   1      0      0      0      0      1
        2 |   1      0      0      0      0      1
        5 |   0      0      0      0      1      1
        6 |   0      0      0      0      0      0
          |
    Total |   7      0      0      0      1      8
----------------------------------------------------

                                              chi2     df     Prob>chi2
------------------------------------------------------------------------
-
Symmetry (asymptotic)                  |      3.00      3         0.3916
Marginal homogeneity (Stuart-Maxwell) |      3.00      4         0.5578
------------------------------------------------------------------------
-

->  kap hep11 hep12 if ep_r == 2

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
  62.50%      54.69%     0.1724     0.1249       1.38      0.0838

->  symmetry hep1_b1 hep1_b2

------------------------------
           |      2 hep1_b
 1 hep1_b |   0      2    Total
----------+-------------------
        0 |    8      0      8
        2 |    2      0      2
          |
    Total |   10      0     10
------------------------------

                                              chi2     df      Prob>chi2
------------------------------------------------------------------------
Symmetry (asymptotic)                  |      2.00      1         0.1573
Marginal homogeneity (Stuart-Maxwell) |      2.00      1         0.1573
------------------------------------------------------------------------
-

->  kap hep1_b1 hep1_b2

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
  80.00%      80.00%     0.0000     0.0000         .           .

->  symmetry hep1_b1 hep1_b2 if ep_r == 2

------------------------------
```

```
          |      2 hep1_b
 1 hep1_b |   0      2    Total
----------+--------------------
        0 |   6      0      6
        2 |   2      0      2
          |
    Total |   8      0      8
------------------------------
```

```
                                                chi2     df      Prob>chi2
------------------------------------------------------------------------------
-
Symmetry (asymptotic)                    |      2.00      1         0.1573
Marginal homogeneity (Stuart-Maxwell) |      2.00      1         0.1573
------------------------------------------------------------------------------
-
```

```
->  kap hep1_b1 hep1_b2 if ep_r == 2

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
  75.00%      75.00%     0.0000     0.0000         .           .
```

```
->  symmetry ttf11 ttf12

-------------------------------------------------
          |                  2 ttf1
   1 ttf1 |   0      1      2      5      6    Total
----------+--------------------------------------
        0 |    8      1      2      0      0     11
        1 |    1      0      0      0      0      1
        2 |    1      0      1      0      0      2
        5 |    0      0      0      0      1      1
        6 |    0      0      3      0      0      3
          |
    Total |   10      1      6      0      1     18
-------------------------------------------------
```

```
                                                chi2     df      Prob>chi2
------------------------------------------------------------------------------
-
Symmetry (asymptotic)                    |      4.33      4         0.3628
Marginal homogeneity (Stuart-Maxwell) |      4.33      4         0.3628
------------------------------------------------------------------------------
-
```

```
->  kap ttf11 ttf12

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
  50.00%      38.89%     0.1818     0.1365       1.33      0.0914
```

```
->  symmetry ttf11 ttf12 if ep_r == 2

-----------------------------------------
```

```
          |              2 ttf1
   1 ttf1 |   0      1      2      6    Total
----------+------------------------------------
        0 |   5      0      1      0      6
        1 |   1      0      0      0      1
        2 |   0      0      0      0      0
        6 |   0      0      1      0      1
          |
    Total |   6      0      2      0      8
-------------------------------------------
```

	chi2	df	Prob>chi2
Symmetry (asymptotic)	3.00	3	0.3916
Marginal homogeneity (Stuart-Maxwell)	3.00	3	0.3916

```
->  kap ttf11 ttf12 if ep_r == 2
```

Agreement	Expected Agreement	Kappa	Std. Err.	Z	Prob>Z
62.50%	56.25%	0.1429	0.1515	0.94	0.1729

```
->  symmetry ttf1b1 ttf1b2

-------------------------------------------
          |              2 ttf1b
  1 ttf1b |   0      1      2      3    Total
----------+------------------------------------
        0 |    9      0      0      0      9
        1 |    5      0      0      0      5
        2 |    2      0      0      0      2
        3 |    2      0      0      0      2
          |
    Total |   18      0      0      0     18
-------------------------------------------
```

	chi2	df	Prob>chi2
Symmetry (asymptotic)	9.00	3	0.0293
Marginal homogeneity (Stuart-Maxwell)	9.00	3	0.0293

```
->  kap ttf1b1 ttf1b2
```

Agreement	Expected Agreement	Kappa	Std. Err.	Z	Prob>Z
50.00%	50.00%	0.0000	0.0000	.	.

```
->  symmetry ttf1b1 ttf1b2 if ep_r == 2
```

```
----------------------------------------
           |              2 ttf1b
  1 ttf1b  |   0      1      2      3    Total
-----------+----------------------------------
         0 |   2      0      0      0      2
         1 |   2      0      0      0      2
         2 |   2      0      0      0      2
         3 |   2      0      0      0      2
           |
     Total |   8      0      0      0      8
----------------------------------------

                                              chi2     df      Prob>chi2
------------------------------------------------------------------------
-
Symmetry (asymptotic)                  |      6.00      3         0.1116
Marginal homogeneity (Stuart-Maxwell) |      6.00      3         0.1116
------------------------------------------------------------------------
-

->  kap ttf1b1 ttf1b2 if ep_r == 2

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
-------------------------------------------------------------------
  25.00%      25.00%     0.0000     0.0000          .           .




->  symmetry neg_b1 neg_b2

----------------------------------------
           |              2 neg_b
  1 neg_b  |   0      1      2      3    Total
-----------+----------------------------------
         0 |  30      0      0      0     30
         1 |  12      0      0      0     12
         2 |   6      0      0      0      6
         3 |   1      0      0      0      1
           |
     Total |  49      0      0      0     49
----------------------------------------

                                              chi2     df      Prob>chi2
------------------------------------------------------------------------
-
Symmetry (asymptotic)                  |     19.00      3         0.0003
Marginal homogeneity (Stuart-Maxwell) |     19.00      3         0.0003
------------------------------------------------------------------------
-
```

```
->  kap neg_b1 neg_b2

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
------------------------------------------------------------------
  61.22%      61.22%     0.0000     0.0000          .           .

->  symmetry neg_b1 neg_b2 if ep_r == 2

-------------------------------------------
          |               2 neg_b
  1 neg_b |   0      1      2      3    Total
----------+--------------------------------
        0 |   17      0      0      0     17
        1 |    6      0      0      0      6
        2 |    6      0      0      0      6
        3 |    1      0      0      0      1
          |
    Total |   30      0      0      0     30
-------------------------------------------

                                          chi2     df      Prob>chi2
------------------------------------------------------------------------
Symmetry (asymptotic)                  |    13.00     3         0.0046
Marginal homogeneity (Stuart-Maxwell) |    13.00     3         0.0046
------------------------------------------------------------------------

->  kap neg_b1 neg_b2 if ep_r == 2

             Expected
Agreement   Agreement     Kappa   Std. Err.         Z      Prob>Z
------------------------------------------------------------------
  56.67%      56.67%     0.0000          .          .           .

. log close
      log:  C:\DATA_10\shehnaz.log
  log type:  text
 closed on:  15 Mar 2010, 09:54:52
------------------------------------------------------------------------
```