POSACONAZOLE ANTIFUNGAL DRUG LEVEL

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CONTENTS

1.0 INTRODUCTION

1.1 Posaconazole is a broad-spectrum triazole agent. It is active against a large number of medically important fungal pathogens including *Candida, Aspergillus, Cryptococcus* and mucoraceous moulds. Posaconazole is available as an oral suspension.

1.2 Posaconazole is used for salvage therapy for aspergillosis, treatment of coccidioidomycosis, chromoblastomycosis, mycetoma or *Fusarium* infections. Posaconazole is also increasingly used as prophylaxis for patients with acute myeloid leukaemia (AML) or myelodysplastic syndrome (MDS) who are expected to become neutropenic, and stem cell transplant recipients receiving immunosuppressive agents.

1.3 In the treatment of invasive fungal infections it is important to use appropriate antifungal therapy to increase the probability of achieving a successful clinical outcome while minimising risks of drug-related toxicity. This requires determination and administration of optimal dose and frequency of the antifungal drug.

1.4 Knowledge of the antifungal drug's pharmacokinetic profile i.e. absorption, metabolism, distribution and elimination; can be problematic as it depends on co-morbidities and immune function of the host. Although concentrations in tissue and body fluids are of greatest interest, serum drug concentrations are accepted as a reasonable substitute.

1.5 The posaconazole bioassay determines the level of posaconazole in patients' serum. The assay will give an indication of whether suitable blood levels have been achieved. Posaconazole concentrations should be measured in the first week of therapy and regularly thereafter.

2.0 SAFETY CONSIDERATIONS

2.1 Standard microbiological technique is adequate for safety whilst preparing the bioassay plate. Gloves and safety goggles should be worn whilst weighing out posaconazole powder. Gloves should be worn whilst handling all patient samples. In cases of high risk specimens, work should be carried out in an exhaust protective cabinet.

2.2 The following Risk and Control of Substances Hazardous to Health (COSHH) Assessments are available, and should be studied for details.

Risk Assessments

No	Title of Risk Assessment
MMMP-RMLM-RISK6	Use of Calipers
MMMP-RMLM-RISK7	The Use of Centrifuges
MMMP-RMLM-RISK8	The Use of Bunsen Burners
MMMP-RMLM-RISK12	Electrical
MMMP-RMLM-RISK13	Use of Exhaust Protective Cabinet
MMMP-RMLM-RISK14	Use of Fine Balance
MMMP-RMLM-RISK15	Fire
MMMP-RMLM-RISK16	Use of Freezers
MMMP-RMLM-RISK17	Use of Fridges
MMMP-RMLM-RISK18	Use of Hot Plate
MMMP-RMLM-RISK19	Use of Incubators

MMMP-RMLM-RISK20	Lone Working
MMMP-RMLM-RISK26	Use of Pipettes
MMMP-RMLM-RISK27	Pregnancy – Safe Working in the Laboratory
MMMP-RMLM-RISK28	Security
MMMP-RMLM-RISK29	Use of Gyratory Shaking Apparatus
MMMP-RMLM-RISK32	Use of Vortex Mixer
MMMP-RMLM-RISK34	Waste Disposal (MRCM)
MMMP-RMLM-RISK35	Use of Water Bath
MMMP-RMLM-RISK36	Use of Multiskan FC Plate Reader
MMMP-RMLM-RISK44	Handling of Human Specimens

COSHH Assessments

No	Title of Assessment
MMMP-RMLM-COSHH21	Bacteriological Agar
MMMP-RMLM-COSHH27	Dimethyl Sulfoxide (DMSO)
MMMP-RMLM-COSHH66	Distel
MMMP-RMLM-COSHH87	Antifungal Drug Powders
MMMP-RMLM-COSHH100	Yeast nitrogen base
MMMP-RMLM-COSHH101	Glucose
MMMP-RMLM-COSHH103	Trisodium Citrate
MMMP-RMLM-COSHH125	Human Plasma

3.0 CROSS REFERENCE

Standard Operating Procedures (SOP's)

No	Title of Procedure
MMMP-RMLM-PROC17	Use of Autoclaves
MMMP-RMLM-PROC21	Use of Calipers
MMMP-RMLM-PROC23	Use of Bunsen Burner
MMMP-RMLM-PROC24	Category 2 organisms
MMMP-RMLM-PROC25	Category 3 organisms
MMMP-RMLM-PROC27	Centrifuge Policy
MMMP-RMLM-PROC29	Disinfection and Decontamination Policy
MMMP-RMLM-PROC31	Use of Drying Cabinet
MMMP-RMLM-PROC33	Use of Exhaust Protective Cabinet
MMMP-RMLM-PROC35	Use of Fine Balance
MMMP-RMLM-PROC38	Use of Freezers
MMMP-RMLM-PROC39	Use of Fridges
MMMP-RMLM-PROC40	Use of Fume Hood
MMMP-RMLM-PROC43	Use of Hot Plate

MMMP-RMLM-PROC45	Use of Incubators
MMMP-RMLM-PROC50	Media Preparation
MMMP-RMLM-PROC57	Use of Pipettes
MMMP-RMLM-PROC61	Safe Handling of Chemicals
MMMP-RMLM-PROC62	Use of Gyratory Shaking Apparatus
MMMP-RMLM-PROC67	Use of Vortex Mixer
MMMP-RMLM-PROC69	Waste Disposal Policy
MMMP-RMLM-PROC70	Use of Water Bath
MMMP-RMLM-PROC76	Results Reporting and Clinical Advice Policy
MMMP-RMLM-PROC78	Retention of Clinical Specimens and Isolates
MMMP-RMLM-PROC79	Sample Acceptance Policy
MMMP-RMLM-PROC80	Data Entry -MRCM
MMMP-RMLM-PROC81	Telephone Policy
MMMP-RMLM-PROC83	MRCM Specimen Reception Procedures
MMMP-RMLM-PROC84	Laminated Laboratory Notices
MMMP-RMLM-PROC98	Leaked Specimen Policy
MMMP-RMLM-PROC99	Personal Protective Equipment
MMMP-RMLM-PROC100	Safety Policy
MMMP-RMLM-PROC105	Quality Control Procedures
MMMP-RMLM-PROC113	Use of Multiskan FC Plate Reader
MMMP-RMLM-PROC128	Booking in Specimens
MMMP-QU-PROC14	Guidance on Uncertainty of Measurement
MMMP-RMLM-FORM1	Work in Progress
MMMP-RMLM-FORM16	Quality Procedure Forms
Q DRIVE	MEDICINE>MYCOLOGY>QPULSE>RMLM>QUALITY>VALIDATIONS
University	Security Policy
University	Fire Policy

4.0 ENVIRONMENTAL CONTROLS

No	Title of Procedure
MMMP-RMLM-PROC86	Environmental Laboratory Monitoring
MMMP-RMLM-FORM9	Incubator Monitoring
MMMP-RMLM-FORM26	Laboratory Temperature Monitoring

5.0 PURPOSE OF THE EXAMINATION

5.1 Serum concentrations of posaconazole should be measured in the majority of patients receiving this drug to ensure that therapeutic concentrations are being achieved. This is necessary as drug absorption can be variable, and levels may be lowered by interactions with other drugs. The assay will give an indication of whether therapeutic blood levels have been achieved.

6.0 PRINCIPLE AND METHOD OF THE PROCEDURE USED FOR EXAMINATION

6.1 Posaconazole levels are measured by bioassay. Bacteriological agar supplemented with yeast nitrogen base with glucose and Trisodium citrate is seeded with *Candida kefyr*. This isolate is susceptible to posaconazole. If present, posaconazole will inhibit the growth of *Candida kefyr*.

6.2 Poasconazole standards are prepared to give concentrations of 0.125 - 7.5mg/L. Patient samples and standards are pipetted into wells cut into the agar and, after incubation, inhibition zone diameters can be measured.

6.3 A standard curve is constructed by plotting mean zone diameter of standards against $\log_{10}$ of drug concentration. Drug concentrations in patient samples can be determined by reference to the standard curve.

7.0 PERFORMANCE CHARACTERISTICS

7.1 Refer to Validation Folder in the Q DRIVE, pathway; MEDICINE > MYCOLOGY > PULSE > RMLM > QUALITY > VALIDATIONS and Measurement of Uncertainty Folder in the Q DRIVE, pathway; MEDICINE > MYCOLOGY > QPULSE > RMLM > QUALITY.

8.0 INTERFERENCES AND CROSS REACTIONS

8.1 Care must be taken when specimen details suggest the patient is receiving another antifungal drug in addition to Posaconazole as this may affect specific and accurate measurement of the Posaconazole drug concentration.
A comment "If this patient is on any other antifungal in addition to posaconazole, this result is invalid" can be used if necessary.

8.2 If the patient sample also contains caspofungin, in combination with posaconazole, *Candida dubliniensis* F/10870 can be used as the seeded organism. The inocoulum should be prepared and adjusted as for section 15.4 but to an OD of 0.060 using the spectrophotometer. All other steps from section 15 should be followed as normal.

8.3 If the patient sample also contains another azole drug, a bioassay may **not** be performed. In such cases HPLC may be necessary.

8.4 Hazy zones may be due to *C. albicans* precipitins, comment "This patient has a minimum level of __ mg/L. However, technical considerations prevent a more accurate result."

8.5 Observation
A good standard curve is not obtained
Cause
Standards are made up incorrectly/ bioassay plate prepared incorrectly/plate read incorrectly.
Action required
Disregard results and repeat bioassay after a careful review of all steps in the bioassay protocol. If possible use new batches of medium, plasma/serum and antifungal drug stock.

8.6 Observation
Internal control is not within 15% of the expected value.
Cause
As above, or internal standard has deteriorated.
Action required
Check previous internal standard results for signs of deterioration. If none are apparent, take action as above.

8.7 Observation
No zone of inhibition produced
Cause
Sample not pipetted into well, or no drug detected.
Action required
Repeat if sample omission suspected. If sample was added to wells ('protein ring' blood residue seen) report level < 0.125 mg/l and comment *NO DRUG DETECTED* (NDD).

If any difficulty is experienced with this test, refer to the lead clinical scientist (or deputy) for advice.

9.0 POTENTIAL SOURCES OF VARIATION

9.1 Transport and storage of samples can affect the integrity of the specimen and cause variability due to degradation of the drug. Samples can be stored at 2 - 8°C for up to 5 days prior to testing. For longer storage, specimens should be stored in the -80°C freezer.

10.0 TYPE OF SAMPLE

10.1 Serum - minimum volume 200µl.
Plasma - minimum volume 200µl.

10.2 Samples may also be in the form of clotted blood (without anti-coagulant), from which plasma/serum should be obtained by spinning the samples at 3000 rpm in a sealed bucket centrifuge for 10 minutes. (Refer to Centrifuge Policy – MMMP-RMLM-PROC27).

10.3 If it is necessary for pharmacokinetic purposes to measure other body fluids, such as urine and cerebrospinal fluid then a HPLC method should be used (see MMMP-RMLM-PROC94; Referral Laboratories).

10.4 **Oral Posaconazole Therapy -** a random sample is required, which can be taken at any time. The first level should be determined one to two weeks after commencement of therapy; this period of time will allow the drug concentration to reach a steady state.
Posaconazole levels should be checked once or twice early in the course of treatment and at later stages to check compliance or possible drug interactions.

10.5 **Specimen transport and storage**
All serum/plasma specimens should be refrigerated prior to testing and a further aliquot should be stored long-term at -80°C. After testing, the original sample is stored in the fridge for a minimum of 1 week (see Retention of Clinical Specimens and Isolates MMMP-RMLM-PROC78). Internal QC specimens should also be retained in the fridge prior to testing.

11.0 TYPE OF CONTAINER AND ADDITIVES

11.1 Serum/plasma in specimen tube

11.2 Clotted blood tube – no EDTA

12.0 EQUIPMENT AND REAGENTS

12.1 Equipment

Hot plate
Water bath
Plastic bioassay plate (Appleton Woods, Catalogue number BC175)
Cork borer No. 3 (8mm holes)
Template for spacing holes
Dial calipers
Semi-logarithmic graph paper (may be required)
Levelling table
Spirit level
Gilson pipettes and sterile pipette tips
Glass universals and bijou bottles (sterile)
Computer programme in Excel
Plate Reader (Thermofisher Scientific – Refer to MMMP-RMLM-PROC113 for use)
Fridge (2-8°C)
-80°C freezer
37°C Incubator

12.2 Reagents

Yeast Nitrogen Base (Difco; Cat. No. 239210) is located in the RMLM chemical cupboard.
Trisodium citrate (Sigma; Cat. No. S4641) is located in the RMLM chemical cupboard.
Glucose powder (Sigma; Cat. No. G7528) is located in the RMLM chemical cupboard.
Base agar (Bacteriological agar (No. 1); Oxoid, Cat. No. L11) is located in the RMLM chemical cupboard.
For preparation of the above media refer to PROC50.
Posaconazole pure substance is obtained from Schering Plough Corporation. It is stored in the RMLM chemical cupboard.
Pooled negative serum/plasma (obtained from Blood Bank) is stored in the -80^{o}C freezer.
Dimethyl sulphoxide (DMSO; Sigma, Cat. No. D-5879) located in the RMLM chemical cupboard.
Sterile distilled water is located in RMLM laboratory.

13.0 CALIBRATION PROCEDURES

13.1 Bi-annual calibration of plate reader, refer to plate reader 'Equipment Folder'.

14.0 QUALITY CONTROL PROCEDURES

Internal Quality Control :

14.1 Control samples with known amounts of posaconazole are placed on each bioassay plate.

14.2 These are prepared as follows:
The 1000mg/L standard (A) is used as a drug stock solution.
This stock is diluted 1:10 (100µL drug stock and 900µL plasma) to give a 100mg/L solution (B).
Internal standard 2mg/L: stock solution B is further diluted 1:50 (50µL drug stock and 2.45ml plasma) to give a 2mg/L standard.
Internal standard 0.75mg/L: stock solution B is diluted 1:10 (100µL drug stock and 900µL plasma) to give a 10mg/L, stock solution (C). (C) is then further diluted 1:13.3 (187.5µL drug stock and 2.3125ml plasma) to give a 0.75mg/L standard.

14.3 Both sets of internal standards are then stored in 200µl amounts in Z5 bottles at -80°C for up to 3 months. There should be enough solution to store 12 aliquots of each internal standard. Ensure the batch folder is completed.

14.4 Internal standards should give a value within a 20% range of the expected value of posaconazole.

14.5 Internal standard values should be logged on to the QC Net. (See MMMP-RMLM-PROC80)

14.6 IQA and EQA samples are set up every month, refer to 'Quality Control' MMMP-RMLM-PROC105.

15.0 PROCEDURAL STEPS

15.1 Prepare stock solution of posaconazole (1000mg/l). Gloves and goggles should be worn. Weigh out pure posaconazole powder into a glass universal and add 10mL of DMSO and vortex vigorously until all of the drug powder has dissolved. The amount of drug powder will depend on drug potency, see Antifungal Drug Preparation MMMP-RMLM-PROC50. This solution may be dispensed into twelve 250µL aliquots in appropriately labelled Z5 bottles and stored at -80°C for up to 3 months. Ensure the batch folder is completed.

15.2 Using the hot plate melt 90ml of base agar before allowing it to cool to 56± 2°C in a warm water bath.

15.3 Put 10ml of concentrated Yeast Nitrogen Base with Glucose and Tri-sodium citrate (YNBG + citrate) solution into the water bath and allow it to warm to the same temperature as the melted base agar.

15.4 Prepare a suspension of *Candida kefyr* San Antonio strain in 7ml of sterile distilled water to less than 0.5 McFarland standard. Measure the optical density of the suspension at a wavelength of 490nm on the spectrophotometer in triplicate, then calculate the average optical density and adjust to 0.026 (approximately $6x10^5$cfu/mL). Vortex the suspension thoroughly before removing 5mL to a new sterile universal to be used as the bioassay inoculum.

15.5 Label a bioassay plate with the antifungal drug to be tested, the date and the initials of the person making the plate. Use the levelling table and spirit level to ensure the bioassay plate is completely flat before mixing and pouring the agar.

15.6 When the agar has cooled to 56± 2°C (allow approximately 30 minutes in the water bath), add the 10ml aliquot of concentrated YNBG + citrate solution and the 5ml *Candida kefyr* suspension (after vortexing it thoroughly). Mix the agar, YNBG + Cit and *Candida kefyr* suspension before pouring it in to the bioassay plate ensuring no bubbles are present. Leave to solidify for at least 30 minutes.

15.7 A set of standards are prepared to allow visualisation of a standard curve. To prepare these first dilute the posaconazole stock solution (A) 1000mg/L in plasma to give the following concentrations:

- 100mg/l - label as concentration B (100µL of 1000mg/L drug stock and 900µL of plasma)
- 10mg/l - label as concentration C (100µL of concentration B and 900µL of plasma)

15.8 Prepare the standards for the standard curve in six bijou bottles as follows:

Bijoux Label	Plasma (µl)	Drug Concentration	Volume of Drug Concentration (µl)	Final Concentration
7.5	1000	A	7.5	7.5mg/L
5	1000	B	50	5mg/L
2.5	1000	B	25	2.5mg/l
1.25	1000	B	12.5	1.25mg/L
0.5	1000	C	50	0.5mg/L

0.25	1000	C	25	0.25mg/L
0.125	1000	C	12.5	0.125mg/L

Ensure a new sterile tip is used for each dilution and all vials are vortexed thoroughly prior to use. Store drug dilutions in the fridge until required.

15.9 When the agar has solidified cut 36 wells of 8mm in diameter with sterile cork borer No.3 (6 rows of 6) using a template as a guide. The plate should then be dried at 37°C, inverted with the lid open, for approximately 1 hour.

15.10 Using the template given, place 40µl of standard, internal control or patient specimen into the appropriate wells. Ensure all standards and samples are at room temperature before use.

15.11 Allow the drug to pre-diffuse until all the wells appear completely dry.

15.12 Incubate the plate at 37^{o}C overnight (approximately 18 hours).

15.13 Measure the diameters of the zones of inhibition around each well using calipers, to the nearest 0.01mm, and record on the results template. Gloves should be worn whilst reading the bioassay plate.

16.0 PRINCIPLE FOR CALCULATING THE RESULTS INCLUDING THE MEASUREMENT UNCERTAINTY OF MEASURED QUANTITY VALUES WHERE RELEVANT

16.1 Calculate the mean diameter for each standard, internal control and patient sample.

16.2 Plot the mean diameters of standards against posaconazole drug concentrations on semi-logarithmic graph paper, with the drug concentrations on the logarithmic ordinate. Draw a straight line of best fit.

16.3 Use the graph to estimate the concentration of drug in the patient specimens and internal controls.

OR

16.4 Calculate using the computer switch on the computer and click on Posa Bioassay icon (Excel computer package) This can be found in the shared folder on the desktop.

16.5 The worksheet will open.

16.6 Enter the results of the standards.

16.7 Before entering the results of the patient specimens you must check to see that the graph is linear and has a correlation of 0.99 or higher. The graph can be viewed at the bottom of the page.

16.8 Enter the internal controls and the patient specimen results into the table including all lab numbers, patient initials and internal standard batch numbers. Up to 4 separate values can be entered for each sample. As the sample values are entered the result will be displayed at the end of the row.

16.9 Once completed go to File, Print, and then click on OK to get a copy of the results and graph. Calculate the percentage difference between the actual internal standard results and the expected results. If the difference between these two values is greater than 15% inform a senior member of staff and repeat the assay.

16.10 Check the printout against the original results record. If all details match then close the worksheet. You will be asked if you want to save changes, press **no**.

16.11 Put the internal standard results into QC net and store the printout in the “Reported” box next to the PC to await authorisation.

16.12 Refer to Measurement of Uncertainty Folder in the Q DRIVE, pathway; MEDICINE > MYCOLOGY > QPULSE > RMLM > QUALITY.

17.0 DETERMINING QUANTITATIVE RESULTS WHEN A RESULT IS NOT WITHIN THE MEASUREMENT INTERVAL

17.1 The measurement interval is 0.125mg/L – 7.5mg/L.
17.2 Levels lower than the 0.125mg/L standard should be reported as <0.125mg/L.
17.3 Results greater than 7.5mg/L should be expressed as >7.5mg/L.

18.0 BIOLOGICAL REFERENCE INTERVALS AND CLINICAL DECISION VALUES (cut off, critical limits)

18.1 A lower target concentration for patients receiving posaconazole for **prophylaxis** of invasive fungal disease is a trough concentration of >0.7mg/L.
18.2 A lower target concentration for patients receiving posaconazole for **treatment** of invasive fungal disease is a trough concentration of >1.3mg/L.

19.0 REPORTABLE INTERVAL OF EXAMINATION RESULTS

19.1 The reportable interval is 0.125mg/L – 7.5mg/L.
19.2 Levels lower than the 0.125mg/L standard, report as <0.125mg/l.
19.3 Results greater than 7.5mg/L should be expressed as >7.5mg/L.

20.0 ALERT/CRITICAL VALUES

20.1 Some results require urgent reporting by telephone (see laminated Laboratory Notices MMMP-RMLM-PROC84). Refer to Telephone Policy MMMP-RMLM-PROC81.

21.0 RESPONSIBILITIES OF PERSONNEL IN AUTHORISING, REPORTING AND MONITORING REPORTS

21.1 Refer to Result Reporting and Clinical Advice Policy (MMMP-RMLM-PROC76) and Data Entry RMLM (MMMP-RMLM-PROC80).
21.2 Using Telepath go into option 21 (Reference Mycology) of the main menu, then option 9 (Assay Miscellaneous).
21.3 Type in specimen number.
21.4 Enter drug as Posaconazole (POSA) into field 1.
21.5 Report as the value of posaconazole obtained in mg/l in the section for the appropriate assay time; pre, post or random.
21.6 Target blood levels mg/L are provided for guidance:
>0.7 prophylaxis,
>1.3 treatment.
21.7 Enter 'level' as cost code.
21.8 Enter phoned through information as required and release for authorisation.

22.0 LABORATORY CLINICAL INTERPRETATION

22.1 Target blood levels mg/L;

>0.7 prophylaxis
>1.3 treatment

22.2 When no drug is detected, add comment "*NO DRUG DETECTED*".

23.0 REFERENCES

British Society for Antimicrobial Chemotherapy Working Party. (1991). Lancet **337**, 1577-1580.

Guidance for Industry- Bioanalytical Method Validation. (2001). Food and Drug Administration.

Treatment of invasive aspergillosis with posaconazole in patients who are refractory to or intolerant of conventional therapy: an externally controlled trial. (2007). Clinical Infectious Diseases **44**, 2-12.

Therapeutic drug monitoring (TDM) of antifungal agents: guidelines from the British Society for Medical Mycology H. Ruth Ashbee, Rosemary A. Barnes, Elizabeth M. Johnson, Malcolm D. Richardson, Rebecca Gorton and William W. Hope.

24.0 APPENDIX

Appendix 1: Posaconazole drug level results sheet
Appendix 2: Specimen template

Appendix 1:

POSACONAZOLE DRUG LEVEL
RESULTS SHEET

Date: _____

Standards (mg/L)	Zone 1 (mm)	Zone 2 (mm)	Zone 3 (mm)
7.5mg/l			
5 mg/l			
2.5 mg/l			
1.25 mg/l			
0.5 mg/l			
0.25 mg/l			
0.125 mg/l			

Samples	Dilution	Lab. No.	Initials	Zone 1 (mm)	Zone 2 (mm)
A					
B					
C					
D					
E					
F					
G					

Appendix 2:

SPECIMEN TEMPLATE

Standards and patient samples are randomised across the bioassay plate, following the template below. This reduces the impact from variability (such as agar thickness) across the plate.

Sample G	Standard 0.125mg/L	Sample D	Standard 2.5mg/L	Sample A	Standard 0.5mg/L
Sample C	Standard 5mg/L	Standard 7.5mg/L	Sample B	Standard 1.25mg/L	Standard 0.25mg/L
Standard 0.125mg/L	Sample E	Standard 5mg/L	Standard 0.25mg/L	Sample D	Standard 2.5mg/L
Sample F	Standard 7.5	Sample G	Standard 1.25mg/L	Standard 0.5mg/L	Sample C
Standard 5mg/L	Standard 1.25mg/L	Standard 2.5mg/L	Standard 0.125mg/L	BLANK	Standard 7.5mg/L
Standard 0.5g/L	Sample B	Standard 0.25mg/L	Sample A	Sample E	Sample F