Document Number:	**SASoM/METHOD/026.v5**
Title:	**'MicroVigene' Software: Analysis**
Version:	**v5**
Author:	**Peter Mullen**

Effective from:	01/02/2021
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SOP History		
Number	Date	Reason for Change
V1	01/02/2013	Original
V2	01/02/2015	Update
V3	01/02/2017	Update
V4	01/02/2019	Update
V5	01/02/21	Update

1.0 Purpose –
This SOP describes the current procedure for performing spot analysis using the MicroVigene software in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to all staff in the SASoM using performing spot analysis using the MicroVigene software.

3.0 Responsibilities –
All staff involved with performing spot analysis using the MicroVigene software are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure

4.0 Procedure –
Launch the MicroVigene software.

From the drop-down menus, select a suitable Template, Image and Platemap.

Find the spots (see SOP SASoM/METHOD/028) and then click on the 'View Dilution Curve' icon.

Click on the Options Icon (the one with the red tick in it).

Select the 'General' tab.
On the left hand side of the window, tick boxes to show the following parameters:

Measurement	Criterion
Y0	100
X0	0, 100
rsu	0
slope	0.1
cv_pct	15
r2	0.9
linear_pct	3
intercept	0

On the right hand side of the window, select the following options:

Intensity	mean net
Group by	replicate average
Normalize	none
Outliers	re-flag
Output	row based
Y0 mode	supercurve (log)

Do not tick the box that says 'One Supercurve'.
Tick the box that says 'RSU relative to SuperCurve EC50.
Make sure that 'Save negative slide' says 'none'.
Make sure that 'Append total protein' says 'none'.

Select the 'Curve Fit' tab.
Select Dilution Curve →Fit Model→4p linear. (If you have 5 sets of samples dilutions then use the 4p model; the 5p model can be used where you have 6 or more sets of dilutions).

Select Outliers→Replicate (2) →Linear Range (3) →Curve Fit (3)
Tick Output→Linear x
Tick output →Linear y
Tick Scale→Auto X-axis
Tick Scale→Auto Y-axis

Click on the 'Montage Curves' icon to show data in graphical form. Check the scales (x & y) and adjust if necessary so that all data points are displayed within the graph area.

Click on 'Save Curves' to keep a copy of the montaged curves.

Click 'Save to File'→'Total Dilution' to save a copy of the data (eg total ERK) without any normalization.

Click 'Save to File'→'Total Protein' to separately assign the data for subsequent normalization purposes.

Having analysed the total protein, close the image and open the paired phospho-protein image.
Find the spots and view dilution curve as previously described. Keep all analysis settings the same as before except that 'Append Total Protein' option is ticked, and the 'Total Protein file' is targeted to the relevant file.

5.0 Personal protection –
A Howie coat must be worn at all times.

6.0 Spillages –
This procedure involves analysis of results, therefore no solutions are involved so no spillages should occur.

7.0 Training –
All staff should under go training in this technique before performing the procedure.

8.0 Related documents –

8.1 MicroVigene User's manual

8.2 SOP SASoM/METHOD/026
MicroVigene' Software: Analysis

8.3 SOP SASoM/METHOD/027
MicroVigene' Software: Platemaps

8.4 SOP SASoM/METHOD/028
MicroVigene' Software: Templates

9.0 Approval and sign off –

Author:

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 11/02/2021

Management Approval:

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 11/02/2021

QA release by:

Name: Alex MacLellan

Position: QA Manager

Signature:  Date: 11/02/2021

STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE