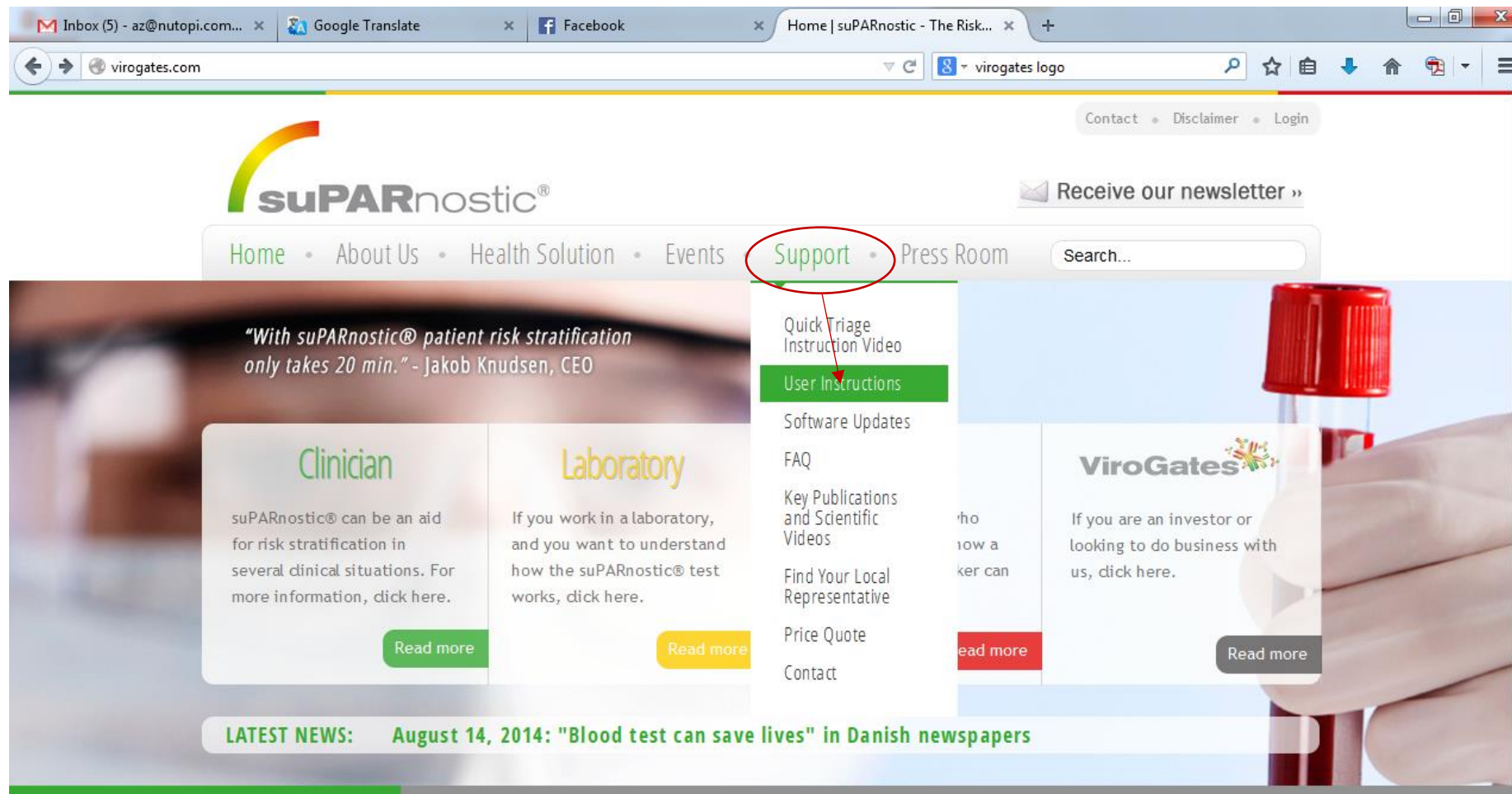




Calculation Template Guide

1. Download the Calculation Template from www.virogates.com website



- Find the *Support* tab on the website

- Choose *User Instructions*

- Find *User Instructions - suPARnostic® ELISA Kit*

- Download *Calculation Tool* by clicking on the green arrow and save the file on a computer

2. Start the suPARnostic[®] Calculation Template file

The screenshot shows the Microsoft Excel interface with the file '20141008 suPARnostic-Calculator V2 (6 in 1) [Protected View]'. The ribbon includes FILE, HOME, INSERT, PAGE LAYOUT, FORMULAS, DATA, REVIEW, VIEW, ADD-INS, HERMA, and ACROBAT. A yellow warning bar at the top states 'PROTECTED VIEW Be careful—files from the Internet can contain viruses. Unless you need to edit, it's safer to stay in Protected View.' and features an 'Enable Editing' button circled in red. The spreadsheet contains several sections: 'Data' (rows 2-4), 'Setup' (rows 9-18), 'Raw data Abs 450 nm' (rows 21-30), and 'Abs - Blank' (rows 33-38). The 'Raw data Abs 450 nm' section has columns 1-12 and rows A-H. The 'Abs - Blank' section has columns 1-12 and rows A-B. The status bar at the bottom shows 'READY' and '75%' zoom.

- Open the file *suPARnostic-Calculator V2 (6 in 1)*
- After opening security warning will occur
- Choose *Enable editing* for broader editing option

3. Preparation of the worksheet – Data section

20141008 suPARnostic-Calculator V2 (6 in 1) - Microsoft Excel

FILE HOME INSERT PAGE LAYOUT FORMULAS DATA REVIEW VIEW ADD-INS HERMA ACROBAT

Clipboard Font Alignment Number Styles Cells Editing

J23

Data

Project:		Lot no:	
Date:		Location:	
Operator:		Room Temp:	

Setup

	1	2	3	4	5	6	7	8	9	10	11	12
A	std a	std a	-	-	-	-	-	-	-	-	-	-
B	std b	std b	-	-	-	-	-	-	-	-	-	-
C	std c	std c	-	-	-	-	-	-	-	-	-	-
D	std d	std d	-	-	-	-	-	-	-	-	-	-
E	std e	std e	-	-	-	-	-	-	-	-	-	-
F	BLANK	BLANK	-	-	-	-	-	-	-	-	-	-
G	Curve C	Curve C	-	-	-	-	-	-	-	-	-	-
H	-	-	-	-	-	-	-	-	-	-	-	-

Measure the Abs at 450 nm. Copy raw data into the empty cells

Raw data Abs 450 nm

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Blank = #DIV/0!

Doublets All Std Doublets 4 Std Doublets 3 Std Singlet All Std Singlet 4 Std Si ...

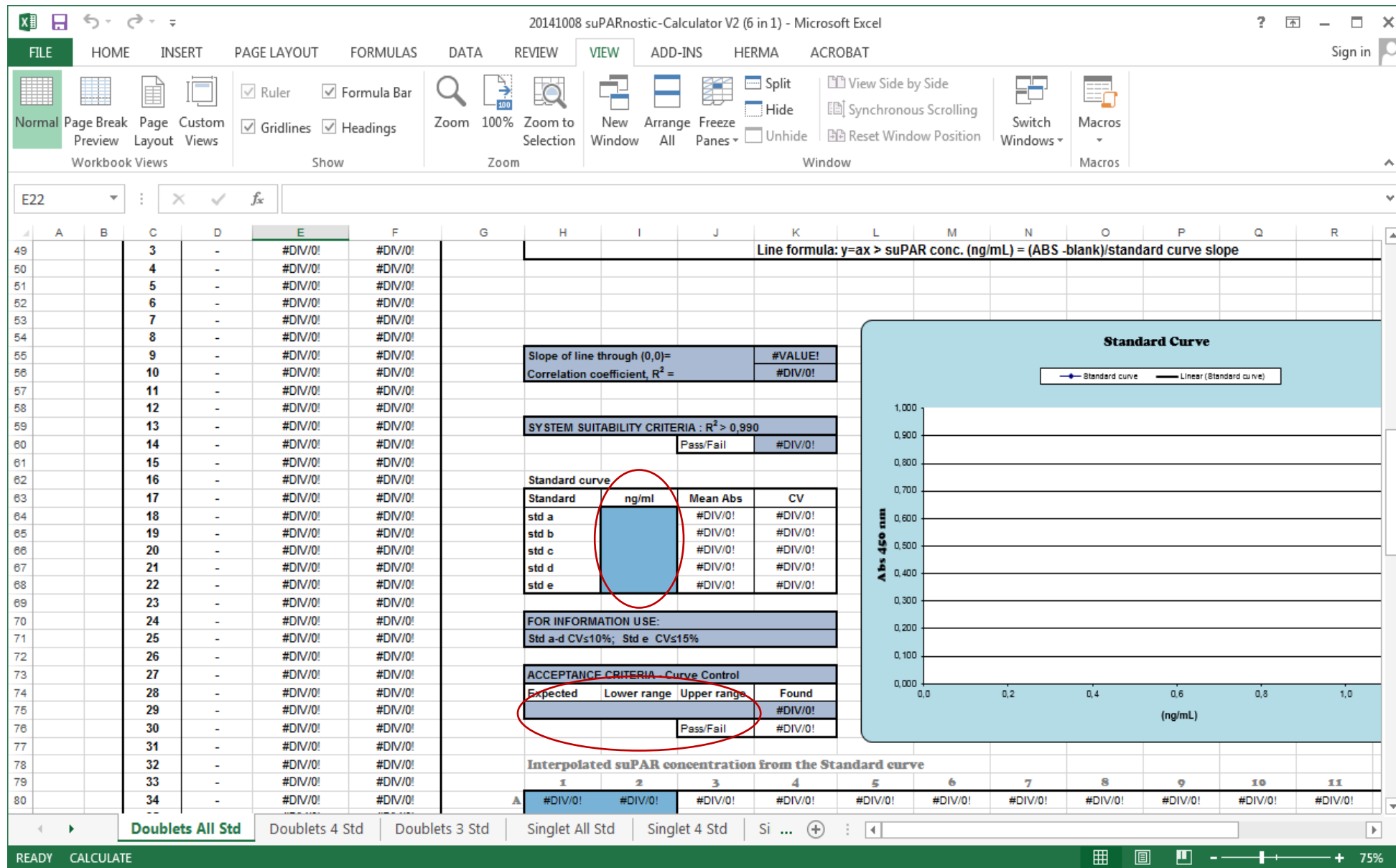
- Depending on which type of the test was chosen, delete sheets which are not going to be used

- Enter the info data in the table in the *Data* section of the sheet

- Enter the plate setup in the *Setup* table below - fill white cells only

- Data from *Setup* will be copied automatically in the *Results* part of the template

3. Preparation of the worksheet – Results section



- Enter the Standards and Curve Control Values which were used in the assay - find it in the Analytical Value Sheet added in each suPARnostic® Kit

- The Standard curve will be seen automatically in the graph after entering Standards concentration values (see step 5)

4. Copy the results

20141027 ERASME ELISA 204LA1-1 raw data - Microsoft Excel

FILE HOME INSERT PAGE LAYOUT FORMULAS DATA REVIEW VIEW ADD-INS HERMA ACROBAT

B2 : 1,295

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1		1	2	3	4	5	6	7	8	9	10	11	12						
2	A	1,295	0,802	0,409	0,185	0,407	0,47	0,254	0,468	0,254	0,265	0	0	450					
3	B	1,044	0,234	0,33	0,586	0,357	0,236	0,325	0,273	0,352	0,467	0	0	450					
4	C	0,738	0,296	0,434	0,497	0,422	0,296	0,285	0,296	0,27	0,262	0	0	450					
5	D	0,448	0,407	0,426	0,684	0,194	0,864	0,349	0,195	0,31	0,34	0	0	450					
6	E	0,139	0,618	0,315	0,196	0,199	0,231	0,167	0,41	0,274	0,257	0	0	450					
7	F	0,06	0,751	0,473	0,206	0,202	0,188	0,384	0,245	0,184	0,192	0	0	450					
8	G	0,313	0,217	0,229	0,672	0,24	0,389	0,185	0,257	0,431	0,073	0	0	450					
9	H	0,408	0,523	0,256	0,307	0,245	0,78	0,402	0,15	0,36	0,072	0	0	450					
10																			
11																			
12																			
13																			
14																			
15																			
16																			
17																			
18																			
19																			
20																			
21																			
22																			
23																			
24																			
25																			

Sheet1 Sheet2 Sheet3

READY AVERAGE: 0,365325 COUNT: 80 SUM: 29,226 100%

- Save Raw Data from ELISA Reader reading as an Excel file

- Copy the table contents

- Paste the results to the *Raw data Abs 450 nm* table in the Calculation template (see step 5)

5. Enter the results into the Calculation Template

20141008 suPARnostic-Calculator V2 (6 in 1) - Microsoft Excel

FILE HOME INSERT PAGE LAYOUT FORMULAS DATA REVIEW VIEW ADD-INS HERMA ACROBAT

Normal Page Break Preview Page Layout Views Custom Views

Workbook Views Show Ruler Formula Bar Gridlines Headings

Zoom 100% Zoom to Selection

New Window Arrange All Freeze Panes Hide Unhide

View Side by Side Synchronous Scrolling Reset Window Position

Switch Windows Macros

E22

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1																		
2			Data		Project:		Lot no:											
3					Date:		Location:											
4					Operator:		Room Temp:											
5																		
6																		
7																		
8					Setup													
9						1	2	3	4	5	6	7	8	9	10	11	12	
10	A	std a	std a			-	-	-	-	-	-	-	-	-	-	-	-	
11	B	std b	std b			-	-	-	-	-	-	-	-	-	-	-	-	
12	C	std c	std c			-	-	-	-	-	-	-	-	-	-	-	-	
13	D	std d	std d			-	-	-	-	-	-	-	-	-	-	-	-	
14	E	std e	std e			-	-	-	-	-	-	-	-	-	-	-	-	
15	F	BLANK	BLANK			-	-	-	-	-	-	-	-	-	-	-	-	
16	G	Curve C	Curve C			-	-	-	-	-	-	-	-	-	-	-	-	
17	H	-	-			-	-	-	-	-	-	-	-	-	-	-	-	
18																		
19																		
20																		
21																		
22																		
23																		
24																		
25																		
26																		
27																		
28																		
29																		
30																		
31																		
32																		

Measure the Abs at 450 nm. Copy raw data into the empty cells

Raw data Abs 450 nm

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Blank = #DIV/0!

Doublets All Std

READY CALCULATE

75%

- Paste the results into the *Raw data 450 nm* table in the Calculation template
- The suPAR concentration will be calculated automatically in the *Results* part of the Table (see step 6)

6. Results interpretation

20141008 suPARnostic-Calculator V2 (6 in 1) - Microsoft Excel

FILE HOME INSERT PAGE LAYOUT FORMULAS DATA REVIEW VIEW ADD-INS HERMA ACROBAT

O17 : fx 42

Results

Sample No.	ID	Mean (ng/mL)	CV
1	1	2.3	0%
2	2	3.2	3%
3	3	3.9	2%
4	4	3.6	4%
5	5	2.1	7%
6	6	5.3	4%
7	7	6.0	6%
8	8	5.9	2%
9	9	4.3	1%
10	10	2.8	3%
11	11	5.0	5%
12	12	4.2	6%
13	13	3.9	0%
14	14	3.5	9%
15	15	2.3	6%
16	16	3.3	5%
17	17	2.1	14%
18	18	4.4	3%
19	19	1.8	13%
20	20	3.4	0%
21	21	2.8	6%
22	22	2.9	4%
23	23	2.5	7%
24	24	2.4	0%
25	25	2.1	1%
26	26	4.0	6%
27	27	2.7	1%
28	28	2.5	1%
29	29	3.9	1%
30	31	3.4	8%
31	32	4.3	0%
32	33	4.0	5%
33	34	4.0	2%
34	35	2.2	1%
35	36	1.7	0%
36	37	2.6	8%
37	38	4.6	1%
38	39	4.8	9%
39	40	5.4	2%
40	41	2.9	6%
41	42	2.7	6%

Trendline formula through (0,0) and the standard curve point is used to calculate the unknown samples

Line formula: $y=ax$ > suPAR conc. (ng/mL) = (ABS -blank)/standard curve slope

Slope of line through (0,0)= 0.0818
Correlation coefficient, $R^2 =$ 1.000

SYSTEM SUITABILITY CRITERIA: $R^2 > 0.999$
Pass/Fail Pass

Standard	ng/ml	Mean Abs	CV
std a	17.6	1.065	3%
std b	13.8	0.840	2%
std c	9.8	0.595	1%
std d	6.3	0.346	3%
std e	0.8	0.073	4%

FOR INFORMATION USE:
Std a-d CVs10%; Std e CVs16%

Expected	Lower range	Upper range	Found
3.2	2.2	4.2	3.8

Pass/Fail Pass

Interpolated suPAR concentration from the Standard curve

	1	2	3	4	5	6	7	8	9	10	11	12
A	17.0	17.6	3.1	3.2	2.8	2.7	4.3	4.4	3.9	4.2	2.2	2.2
B	13.5	13.8	4.0	3.9	5.2	4.9	1.7	2.0	2.8	2.7	1.7	1.7
C	9.7	9.6	3.7	3.5	4.3	4.0	3.4	3.4	2.5	2.5	2.4	2.7
D	5.5	5.7	2.0	2.3	3.8	3.9	2.9	2.7	3.9	3.8	4.5	4.6
E	1.2	1.1	5.1	5.5	3.3	3.7	2.8	3.0	3.2	3.6	4.5	5.1
F	0.0	0.0	5.8	6.3	2.2	2.4	2.4	2.6	4.3	4.3	5.5	5.4
G	3.6	3.7	5.8	6.0	3.4	3.1	2.4	2.4	3.9	4.2	3.0	2.8
H	2.3	2.3	4.3	4.3	1.9	2.4	2.1	2.1	3.9	4.0	2.5	2.8

Standard Curve

Standard curve

READY CALCULATE

Doublets All Std

- The suPAR concentration from samples are calculated automatically and can be seen in the *Results* section in the left table

- Cells with criteria to accept the results will be filled automatically – this will be: Correlation coefficient R^2 , Standards CV%, Curve control concentration

- If the results met the criteria, Excel automatically fills the cell with ,Pass' and green color background; if not Excel fills it with ,Fail' and pink background