

## **APPENDIX 4 (SEROLOGICAL METHODS)**

### *4.2.1a) List of requirements for Bartonella IFA as per Focus Diagnostics\* kit protocol*

#### Reagents included in the kit:

♣ <i>Bartonella</i> IFA substrate slides	→	Ag
♣ IgM / IgG conjugate – dual species	→	CONJ IgM / IgG
♣ Polyvalent positive control	→	CONTROL +
♣ Negative control	→	CONTROL -
♣ IgM / IgG pretreatment diluent	→	DIL IgM / IgG
♣ Mounting medium	→	REAG MONT
♣ PBS	→	BUF

#### Materials required but not included in the kit:

- ♣ 24 x 50 mm coverslips
- ♣ Microcentrifuge tubes or microtiter plate for serum dilutions
- ♣ Test tube rack
- ♣ 35 to 37 °C incubator or water bath for slide incubation
- ♣ 2 – 20 µl pipette
- ♣ 20 – 200 µl pipette
- ♣ Pipette tips
- ♣ Coplin jars or slide staining dish with slide holder
- ♣ Humid chamber for incubation
- ♣ Distilled water
- ♣ Timer
- ♣ Filter paper for blotting the slides
- ♣ Fluorescent microscope

#### Kit warnings:

- ♣ The source of kit reagents and the materials from which the products (including the negative controls), were derived have been screened for Hepatitis B surface antigens, Hepatitis C antibody and HIV-1/2 (AIDS) antibody by FDA-approved methods and have been found to be negative, however no test method can provide complete certainty.
- ♣ Do not use hyperlipaemic, haemolysed, heat inactivated or contaminated serum as this may give erroneous results.
- ♣ Do not substitute or mix reagents from different kit lots or from other manufacturers.
- ♣ Use only protocols described within the package insert.

#### 4.3.1a) HIV-positive patient results according to age group categorization

##### HIV-POSITIVE (14 – 25 YRS)

IFA (IgG) results	IFA (IgM) results				Grand Total	
	Negative	Positive ( <i>B. henselae</i> & <i>B. quintana</i> )	Positive ( <i>B. henselae</i> )	Positive ( <i>B. quintana</i> )		
Negative	10	4	-	-	14	63.6%
Positive ( <i>B. henselae</i> & <i>B. quintana</i> )	4	-	-	-	4	18.2%
Positive ( <i>B. henselae</i> )	1	-	-	-	1	4.5%
Positive ( <i>B. quintana</i> )	2	-	-	1	3	13.6%
<b>Grand Total</b>	<b>17</b>	<b>4</b>	<b>-</b>	<b>1</b>	<b>22</b>	
	77.3%	18.2%		4.5%		

##### HIV-POSITIVE (26 – 35 YRS)

IFA (IgG) results	IFA (IgM) results				Grand Total	
	Negative	Positive ( <i>B. henselae</i> & <i>B. quintana</i> )	Positive ( <i>B. henselae</i> )	Positive ( <i>B. quintana</i> )		
Negative	73	6	2	2	83	65.4%
Positive ( <i>B. henselae</i> & <i>B. quintana</i> )	17	3	1	2	23	18.1%
Positive ( <i>B. henselae</i> )	11	3	1	-	15	11.8%
Positive ( <i>B. quintana</i> )	5	-	-	1	6	4.7%
<b>Grand Total</b>	<b>106</b>	<b>12</b>	<b>4</b>	<b>5</b>	<b>127</b>	
	83.5%	9.4%	3.1%	3.9%		

##### HIV-POSITIVE (36 – 45 YRS)

IFA (IgG) results	IFA (IgM) results				Grand Total	
	Negative	Positive ( <i>B. henselae</i> & <i>B. quintana</i> )	Positive ( <i>B. henselae</i> )	Positive ( <i>B. quintana</i> )		
Negative	93	4	2	2	101	72.7%
Positive ( <i>B. henselae</i> & <i>B. quintana</i> )	20	4	1	-	25	18%
Positive ( <i>B. henselae</i> )	7	-	1	-	8	5.8%
Positive ( <i>B. quintana</i> )	4	-	1	-	5	3.6%
<b>Grand Total</b>	<b>124</b>	<b>8</b>	<b>5</b>	<b>2</b>	<b>139</b>	
	89.2%	5.8%	3.6%	1.4%		

##### HIV-POSITIVE (46 – 55 YRS)

IFA (IgG) results	IFA (IgM) results				Grand Total	
	Negative	Positive ( <i>B. henselae</i> & <i>B. quintana</i> )	Positive ( <i>B. henselae</i> )	Positive ( <i>B. quintana</i> )		
Negative	36	3	2	1	42	63.6%
Positive ( <i>B. henselae</i> & <i>B. quintana</i> )	14	2	-	1	17	25.8%
Positive ( <i>B. henselae</i> )	4	-	1	-	5	7.6%
Positive ( <i>B. quintana</i> )	2	-	-	-	2	3.0%
<b>Grand Total</b>	<b>56</b>	<b>5</b>	<b>3</b>	<b>2</b>	<b>66</b>	
	84.8%	7.6%	4.5%	3.0%		

**HIV-POSITIVE (>55 YRS)**

IFA (IgG) results	IFA (IgM) results				Grand Total	
	Negative	Positive ( <i>B. henselae</i> & <i>B. quintana</i> )	Positive ( <i>B. henselae</i> )	Positive ( <i>B. quintana</i> )		
Negative	10	1	-	-	11	73.3%
Positive ( <i>B. henselae</i> & <i>B. quintana</i> )	2	1	-	-	3	20%
Positive ( <i>B. henselae</i> )	-	-	-	-	-	
Positive ( <i>B. quintana</i> )	1	-	-	-	1	6.7%
<b>Grand Total</b>	<b>13</b>	<b>2</b>	<b>-</b>	<b>-</b>	<b>15</b>	
	86.7%	13.3%				

4.3.1b) Summary of IgM percentages for each age group:

Ages:	Positive ( <i>B. henselae</i> & <i>B. quintana</i> )	Positive ( <i>B. henselae</i> )	Positive ( <i>B. quintana</i> )	Total positives:
14 – 25	18.2%	-	4.5%	22.7%
26 – 35	9.4%	3.1%	3.9%	16.4%
36 – 45	5.8%	3.6%	1.4%	10.8%
46 – 55	7.6%	4.5%	3.0%	15.1%
>55	13.3%	-	-	13.3%

4.3.1c) Summary of IgG percentages for each age group:

Ages:	Positive ( <i>B. henselae</i> & <i>B. quintana</i> )	Positive ( <i>B. henselae</i> )	Positive ( <i>B. quintana</i> )	Total positives:
14 – 25	18.2%	4.5%	13.6%	36.3%
26 – 35	18.1%	11.8%	4.7%	34.6%
36 – 45	18%	5.8%	3.6%	27.4%
46 – 55	25.8%	7.6%	3.0%	36.4%
>55	20%	-	6.7%	26.7%