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Bartonella species in human and animal populations in Gauteng, South Africa, 2007-2008

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APPENDIX 4 (SEROLOGICAL METHODS)

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

Reagents included in the kit:

- ***Bartonella* 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: Negative	Positive (*B. henselae* & *B. quintana*)	Positive (*B. henselae*)	Positive (*B. quintana*)	Grand Total	
Negative	**10**	**4**	**-**	**-**	**14**	63.6%
Positive (*B. henselae* & *B. quintana*)	**4**	**-**	**-**	**-**	**4**	18.2%
Positive (*B. henselae*)	**1**	**-**	**-**	**-**	**1**	4.5%
Positive (*B. quintana*)	**2**	**-**	**-**	**1**	**3**	13.6%
Grand Total	**17**	**4**	**-**	**1**	**22**	
	77.3%	18.2%		4.5%		

HIV-POSITIVE (26 – 35 YRS)

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

HIV-POSITIVE (36 – 45 YRS)

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

HIV-POSITIVE (46 – 55 YRS)

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

HIV-POSITIVE (>55 YRS)

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

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

Ages:	Positive (***B. henselae* & *B. quintana***)	Positive (***B. henselae***)	Positive (***B. quintana***)	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 (***B. henselae* & *B. quintana***)	Positive (***B. henselae***)	Positive (***B. quintana***)	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%