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6 **Methods for real-time PCR-based diagnosis of *Chlamydia pneumoniae*,**
7 ***Chlamydia psittaci* and *Chlamydia abortus* infections in an opened molecular**
8 **diagnostic platform**

9
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24 **Summary**

25

26 The advances in molecular biology of the last decades have dramatically improved the field of
27 diagnostic bacteriology. In particular PCR-based technologies have impacted the diagnosis of
28 infections caused by obligate intracellular bacteria such as pathogens from the *Chlamydiaceae*
29 family. Here, we describe a real-time PCR-based method using the Taqman technology for the
30 diagnosis of *Chlamydia pneumoniae*, *Chlamydia psittaci* and *Chlamydia abortus* infection. The
31 method presented here can be applied to various clinical samples and can be adapted on opened
32 molecular diagnostic platforms.

33

34 **Key words:** *Chlamydia pneumoniae*, *Chlamydia psittaci*, *Chlamydia abortus*, molecular
35 diagnostic, real-time PCR, DNA extraction, Taqman

36 **1. Introduction**

37 *Chlamydiae* are obligate intracellular bacteria, among which several species are pathogenic
38 towards humans and can cause a broad range of diseases. *Chlamydia trachomatis* is involved in
39 urogenital infection as well as ocular, joint and oropharyngeal infections. *Chlamydia*
40 *pneumoniae* and *Chlamydia psittaci* are primarily associated with community-acquired
41 pneumonia (CAP). *C. pneumoniae* have been essentially associated with infections in humans [1]
42 but some studies suggest an association with other mammals such as koalas [2-8]. *C.*
43 *pneumoniae* might also be a causative agent of an asthma-like syndrome in children [9]. *C.*
44 *psittaci* is the etiologic agent of a respiratory zoonosis transmitted by birds, in particular parrots
45 and parakeets, but also chicken from the food industries as well as feral pigeons [2]. *C. abortus* is
46 genetically closely related to *C. psittaci* but displays a distinct animal and tissue tropism as it can
47 colonize the placenta of cows, goats, cattles, pigs and horses, which can lead to abortion. *C.*
48 *abortus* can be transmitted to humans, for instance through the exposure to infected animal
49 abortive tissues, with the same outcome for pregnant women [2].

50 The diagnosis of infections due to intracellular bacteria has been dramatically improved by PCR-
51 based methods developed in the last decades. This is particularly true for the diagnosis of *C.*
52 *trachomatis*, for which several commercial systems have been developed. The laboratory of
53 molecular diagnostic of the Institute of Microbiology of the Lausanne University Hospital
54 (Lausanne, Switzerland), developed a molecular diagnostic platform recently described by
55 Greub and colleagues, which allows one to perform multiple PCRs simultaneously targeting
56 different pathogens, thanks to the standardisation of the parameters (i.e. amplicon length, and
57 primers and probes T_m) of the reactions [10]. This molecular platform based on the Taqman-
58 probes technology (Applied Biosystems) allows for performance of up to 91 different PCR
59 reactions corresponding to 69 pathogens and/or resistance genes in a single microplate [10].
60 Among them are a real-time PCR for the detection of *C. pneumoniae* [11] and a real-time duplex
61 PCR for the detection of *C. psittaci* and *C. abortus* infection from various clinical samples
62 (Table 1), both based on the Taqman probe technology [12]. The *C. pneumoniae* PCR is based
63 on the *pst1* gene and the *C. psittaci*-*C. abortus* duplex PCR was designed as follows: 1) PCR1
64 targets a DNA sequence of the 16S–23S rRNA operon allowing the detection of both *C. psittaci*
65 and *C. abortus* and 2) PCR 2 targets the coding DNA sequence CPSIT_0607 so far unique to *C.*
66 *psittaci* [12]. In this chapter, the methods to achieve the *C. pneumoniae* PCR and the *C. psittaci*-

67 *C. abortus* duplex PCR on an opened molecular diagnostic platform are detailed. For this
68 specific chapter, part of the samples' processing methods and infrastructure descriptions have
69 been extracted from the accredited documentation of the Laboratory of Diagnostic of the
70 Institute of Microbiology of the University of Lausanne.

71

72

73

74

75 **2. Materials**

76 **2.1 Laboratory organization**

77 All the procedures should be carried-out according to molecular diagnostic principles aimed to
78 avoid contaminations with microorganisms or nucleic acids ([See Note 1](#)).

79

80 **2.2 Sample processing**

81 1. N-acetyl cysteine solution: in a 50 ml conical tube, containing 1.0 g of N-acetyl cysteine,
82 add 50 ml of Tris-Sodium-Citrate-di-hydrate buffer. The solution should be used the
83 same day.

84 2. Molecular biology grade PBS: DNase-Free, RNase-Free, does not contain detectable
85 amounts of nucleic acid or any nucleic-acid extractions' compatible molecular biology
86 grade solution.

87

88 **2.3 Material for DNA extraction**

89 1. Several automated instruments exist for DNA extraction, in the Institute of microbiology,
90 nucleic-acid extraction is achieved with the MagNA Pure 96^R instrument (Roche): an
91 automated system for the extraction of nucleic-acid from bacteria (as well as other
92 microorganisms such as viruses) using a technology based on magnetic glass particles.
93 The MagNA Pure 96^R instrument reagents are those provided by the manufacturer. This
94 nucleic-acid extraction instrument has been associated with a liquid handling distribution
95 system, the STARlet^R instrument (Hamilton^R) [13] ([See Note 2](#)).

96

97 2. Molecular biology grade PBS: DNase-Free, RNase-Free, does not contain detectable
98 amounts of nucleic acid or any nucleic-acid extractions' compatible molecular biology
99 grade solution.

100

101 **2.4 Assembly of the PCR plate and amplification**

102

103 1. TaqMan Universal Master Mix (Applied Biosystems).

104 2. Primers and probes (Table 1).

- 105 3. Molecular biology grade water: DNase- and RNase-free water, which does not contain
106 detectable amounts of nucleic acid.
- 107 4. Positive control. The positive controls consist of synthetic plasmids containing the exact
108 PCR amplicon [12,11].
- 109 5. PCR instrument ([see Notes 3 and 4](#)).

110

111 **3. Methods**

112

113 **3.1 Sample processing**

114 In clinical practice, the detection of *C. pneumoniae*, *C. psittaci* and *C. abortus* can be achieved
115 from a broad range of samples (Table 2). Depending on the nature of the sample (liquid, viscous
116 or solid) a specific processing will be required either to concentrate (liquid samples) or to
117 homogenize (viscous or solid samples) the specimen.

118

- 119 1. Introduce the lab request into the laboratory information system (LIS) to generate the
120 barcode labels necessary to manage tubes during the analytic process ([See Note 5](#)).
- 121 2. For each sample, label three screw cap tubes (2ml) with the barcode as follows: one
122 “native” tube, one “native-aliquot” tube and one “DNA tube”.
- 123 3. Under the laminar flow dedicated for molecular diagnostics in the “specimen-receiving
124 laboratory,” distribute the sample into the native-aliquot tube and the native tube. The
125 native tube will be frozen at -80°C and kept as backup in case of need. If necessary
126 several native tubes can be stored; a suggestion for biopsies would be to store both the
127 native samples and the processed samples in distinct tubes. The aliquot-native tube
128 together with the DNA tube will be transported to the molecular diagnostic laboratory
129 and used for nucleic acids extraction. Viscous samples such as respiratory secretions
130 should be homogenized by liquefaction where as solid samples such as biopsies and
131 fragments should be crushed as described below.
- 132 4. Liquefaction of viscous secretions: respiratory secretions (sputum, bronchial aspirate,
133 stomach tube) for the detection of *C. pneumoniae* and *C. psittaci* can be too viscous to be
134 homogenized by simple vortexing. Liquefaction can be achieved using N-acetyl-L-
135 cysteine, a reducing substance having free thiol groups (-SH), to reduce specimen

136 viscosity by breaking the disulfide bonds of glycoproteins that constitute the bronchial
137 mucus. Any other viscous fluids shall also be liquefied. To do so, visually control the
138 viscosity of the sample; if liquefaction is necessary, transfer a suitable amount of the
139 sample in a 15 or 50 ml conical tube using a single use plastic Pasteur pipette, under the
140 hood with laminar flow. Add an equal amount of N-acetyl-L-cysteine solution. A larger
141 amount of N-acetyl-L-cysteine solution can be needed for very sticky samples. Vortex
142 and visually check the liquefaction of the sample and if necessary, leave the sample on a
143 rotor for up to 30 minutes. At the end of the incubation, visually check the liquefaction of
144 the sample and centrifuge the sample 30 minutes at 3000 g then remove the supernatant
145 using a pipette with filter tips, leaving 1 to 2 mL of liquid in which the pellet will be
146 resuspended. Transfer the amount necessary for nucleic acids extraction (more than 200
147 μ l) into the tube labelled as aliquot-native" ([See Note 6](#)), and keep the rest in the tube
148 labelled as "native". Store the native tube at -80°C in the core specimen receiving
149 laboratory and transport the aliquot-native tube and the DNA tube to the molecular
150 diagnostic laboratory.

151 5. Crushing of solid specimens. Solid samples such as biopsies and fragments that cannot be
152 homogenized either using the vortex or by liquefaction should be crushed as follows.
153 Under the hood with laminar flow put the little pieces in the crushing device with part of
154 the solution of the native sample (if any) or add a solution adapted to the DNA extractor
155 (PBS) using a pipette with filtered tips ([see Note 7](#)). If the specimen is too big, take a
156 piece of it with sterile forceps and place it in a sterile Petri dish to cut it into several small
157 pieces. Crush a piece of the specimen and keep the rest in reserve. Start the crushing
158 device. Generally, the fragments are successfully crushed, if not, this procedure is still
159 sufficient to release microorganisms from the specimen by compression. In this case,
160 avoiding some remaining large fragments, transfer the crushed sample (volume) in the
161 aliquot-native tube, and the rest in the native tube for storage at -20°C. If necessary add
162 some molecular biology grade water into the aliquot-native tube to reach the minimum
163 volume suitable for nucleic acids extraction. Vortex the tube. Transport the aliquot-
164 native tube and the DNA tube to the molecular diagnostic laboratory.

165

166 **3.2 DNA extraction**

- 167 1. Using the STARlet^R liquid handling instrument, transfer 200 ul of the sample tube in the
168 extraction plate of the MagNA Pure 96^R instrument.
- 169 2. Extraction control (EC). The EC corresponds to a tube submitted to the same extraction
170 protocol that the clinical specimen but in which the volume of the clinical sample is
171 replaced by an equal volume of a solution adapted to the DNA extractor (PBS). An
172 extraction control is needed for each run of extraction ([see Note 8](#)). The extraction
173 control must be negative when the PCR specific for the pathogen (s) tested is (are) made.
- 174 3. Transfer the 96-well microplate into the MagNAPure 96^R instrument and start the nucleic
175 acids extraction program according to the user manual. Nucleic acids can be eluted either
176 in 50µl or 100µl of elution buffer depending on the chosen program and are maintained
177 at 4°C ([See Note 9](#)).

178

179 **3.3 Preparation of the PCR controls**

- 180 1. Negative control of PCR. Each run of amplification should contain a PCR negative
181 control that consists of the extraction control used as a template. The negative control is
182 used to test the reactivity of the component of the reaction mixture. It should not be
183 contaminated with target DNA and should not allow non-specific amplification.
- 184 2. Positive control. Positive controls consist of synthetic plasmid DNA containing the target
185 sequence of the PCRs [12,11] ([See Note 10](#)). For each run of PCR, three reactions with
186 three dilutions of the positive controls containing 10, 10² and 10³ DNA copies per
187 reaction should be added. They will serve both to generate the standard curve that will be
188 used for the quantification of the positive control and to determine the sensitivity of the
189 reaction based on the positive amplification, the reaction containing 10 copies of DNA.
- 190 3. Inhibition control. The presence of PCR inhibitors should be tested for each sample. To
191 do so the inhibition control reaction consists of a reaction in which 200 copies of the
192 positive control is added to the reaction mixture containing the DNA specimen to be
193 tested [10].

194

195 **3.4 Preparation of the PCR mix, design and assembly of the PCR microplate and** 196 **amplification**

- 197 1. Design of the Taqman PCR plate. This can be achieved using dedicated software such as
198 the SDS 2.4.1 software (Applied Biosystems) allowing the design of either 96 or 384 well
199 PCR plates [10]. The same final volume (20 µl) is convenient for both type of plate. It is
200 strongly recommended to do each analysis in duplicate (or even in triplicate). When an
201 internal control is not used, an additional well for the inhibition control is needed, if not
202 using internal inhibition control. Moreover, there is a need for at least one well for the
203 inhibition control, one well for the negative control and 3 wells for the standard curve. A
204 standard curve is required for each PCR. One regression curve is required for the PCR1
205 and another for the PCR2 [12].
- 206 2. In the DNA-free laboratory, prepare the PCR mix. A single mix is needed for the
207 detection of *C. psittaci* and *C. abortus*. In a final volume of 20 µl, add 5 µl of the
208 extracted DNA, the forward and reverse primers and the probes at the final concentration
209 indicated in table 2. The inhibition control reaction consists of the same reaction in
210 which, 200 copies of the control plasmids are added. As an extraction negative control
211 the DNA is replaced by the same volume of the extraction control. As a negative control
212 of the PCR, the DNA is replaced by molecular grade water.
- 213 3. Assembly of the PCR plate. The assembly of the PCR plate can be done manually for 96
214 well plates ([See Note 11](#)) or using automated instruments for 384 well plates [10]. The
215 reactions are achieved in a final volume of 20 µl with 5 µl of DNA sample [12,11].
- 216 4. Amplification. Run the ABI 7900 instrument or similar thermocycler using the following
217 cycling conditions: 2 min at 50°C, 10 min at 95°C followed by 45 cycles of 15s at 95°C
218 and 1min sec at 60°C.

219

220 **3.5 Interpretation of the results**

221 The SDS 2.4.1 software is used for analysis and interpretation of the results.

222 The results (qualitative and quantitative) are then checked and introduced into the LIS system.

223 At the end of the process the final results are validated by a clinical microbiologist.

224

- 225 1. Analyze the results of the positive controls with adequate software [10].
- 226 2. Internal quality control. For an analysis to be valid: a) the run should pass the internal
227 quality control and b) the positive controls 10 copies must be detected.

- 228 3. Negative controls. Control that there is no amplification in the negative control reaction.
- 229 4. Inhibition control. Control that there is amplification in the inhibition control tube
230 containing 200 copies of the positive control plasmid (at least of 50 copies).
- 231 5. Positive result. A result is positive if the fluorescence reaches the threshold automatically
232 set by the software or manually set by the user according to the instrument.
- 233 6. Interpretation of the *C. psittaci*-*C. abortus* PCR ([See Note 12](#)).
- 234 7. If you suspect a contamination, when for instance only 1 reaction out of 3 reactions is
235 positive or when amplification occurs in the extraction control or in the PCR negative
236 control, you can follow the procedure described by Greub and colleagues for these
237 situations [10].

238

239 **4. Notes**

240 *Note 1*

241 As described by Greub and colleagues it is important to organize the laboratory of molecular
242 diagnostic in different rooms/spaces corresponding to pre-amplification and post-amplification
243 area [10]. Moreover, the processing of clinical samples in the laboratory where the samples are
244 received should be achieved under a laminar flow dedicated for molecular diagnostics that is
245 distinct from laminar flows dedicated for conventional culture based microbiology diagnostics.
246 Sterile samples should also be processed in dedicated laminar flows. The use of disposable lab
247 coats, gloves and pipettes with filter tips is recommended. Thus, we recommend the following
248 infrastructures and instruments: **A)** the sample reception laboratory should be equipped with a
249 hood with laminar flow and UV, a vortex, a crushing instrument, a centrifuge, disposable lab
250 coats and gloves, pipettes and filter tips, 2ml screw cap micro-tubes, sterile forceps and sterile
251 Petri dishes; **B)** the nucleic acids' extraction laboratory should also be equipped with a hood with
252 laminar flow and UV, a vortex, pipettes and filter tips, disposable lab coats and gloves without
253 mineral powders such as talc to prevent any deposition in the extraction tubes; **C)** the PCR
254 master-mix should be prepared in a DNA free laboratory equipped with a hood with UV but
255 without laminar flow; for all the post extraction area, hood with laminar flow should be
256 prohibited in order to avoid the deposition of nucleic acids (NA) in opened tested tubes. In
257 addition, the following instruments should be available in this laboratory: a vortex, a micro-
258 centrifuge, micro-tubes (0.2, 0.7 and 1.5 ml), 2ml screwed tube, pipettes and filter tips (0-10, 2-

259 20, 20-200 et 200-1000 µl); **D)** the positive control should be prepared and stored in a “DNA
260 laboratory” equipped with a hood with UV but without laminar flow, a vortex, a micro-
261 centrifuge, micro-tubes, pipettes and filter tips; **E)** It is recommended to assemble the PCR plate
262 a dedicated pre-amplification laboratory different from the positive control laboratory.

263

264 **Note 2:**

265 If processing a large number of samples, it is recommended to select automated distribution
266 systems that can recognize bar-coded tubes.

267

268 **Note 3:**

269 The assembly of the PCR plate can be achieved manually for 96 well plates or alternatively can
270 be done by automated instruments to increase the precision and to avoid mistakes, especially
271 when preparing 384 well plates [10].

272

273 **Note 4:**

274 The PCR conditions described herein for the detection of *C. pneumoniae*, *C. psittaci* and *C.*
275 *abortus* have been optimized for the PCR instrument ABI 7900 HT (Applied Biosystems) [12].
276 The reagent concentrations and the amplification program should be adapted if using other
277 instruments [10].

278

279 **Note 5:**

280 Tubes management is crucial all along the analytic process to avoid major errors such as
281 inversions or contaminations. We strongly recommend the use of barcoded tubes.

282

283 **Note 6:**

284 If using an automated system such as the STARlet^R instrument (Hamilton^R), do not forget to
285 provide the dead volume of the device.

286

287 **Note 7:**

288 To avoid the dilution of the specimen, which could negatively impact the sensitivity of the PCR,
289 do not add too much solution for the crushing of the sample.

290

291 **Note 8:**

292 We recommend placing the extraction control at the end of the series where contamination are
293 more expected to occur rather than at the first position of a series.

294

295 **Note 9:**

296 It is recommended to store the DNA sample at -80 ° C.

297

298 **Note 10:**

299 The concentration (DNA copies per ml) of the positive control should be precisely determined.

300 The stock solution of the positive control should be stored in a separate, dedicated room.

301

302 **Note 11:**

303 The assembly of the PCR plate can be achieved manually especially for 96 well plates. In the
304 DNA free laboratory prepare the PCR mix without the DNA. Place a 96-well micro-plate on a
305 chilled metal rack and distribute the PCR master mix in the 96-well plate according to the
306 Taqman plate set-up. Add the molecular grade water to the corresponding wells (*i.e* in the
307 negative controls) and transfer the plate with the chilled metal plate in the assembly laboratory.

308 In the assembly laboratory, add the DNA samples starting with the extraction control to avoid
309 any contamination at this stage. Close the tubes when the DNAs of a patient are pipette in order
310 to avoid contamination by aerosols. Repeat this for all the patients' DNAs. When all the patients'
311 DNAs are added, cover the corresponding wells and carefully add the positive control in the
312 wells corresponding to the "inhibition control". It is important to avoid any contamination of the
313 patients' test-tube with the positive controls which would lead to false positive results. Exit the
314 room with the plate in a chilled metal rack and transfer it to the amplification room where the
315 thermocycler is located.

316

317 **Note 12:**

318 As described in Opota et al 2015, if both PCR are positive (PCR1 and PCR2) in a respiratory
319 sample, this indicates the presence of *C. psittaci* DNA [12]. Samples positive for PCR1 and

320 negative for PCR2 can be considered as positive for *C. psittaci* for respiratory specimens and
321 positive for *C. abortus* for genital specimens.

322

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326

327 **References**

- 328 1. Lamoth F, Greub G (2010) Fastidious intracellular bacteria as causal agents of community-
329 acquired pneumonia. Expert review of anti-infective therapy 8 (7):775-790.
330 doi:10.1586/eri.10.52
- 331 2. Longbottom D, Coulter LJ (2003) Animal chlamydioses and zoonotic implications. Journal of
332 comparative pathology 128 (4):217-244
- 333 3. Yin L, Kalmar ID, Lagae S, Vandendriessche S, Vanderhaeghen W, Butaye P, Cox E,
334 Vanrompay D (2013) Emerging Chlamydia psittaci infections in the chicken industry and
335 pathology of Chlamydia psittaci genotype B and D strains in specific pathogen free chickens.
336 Veterinary microbiology 162 (2-4):740-749. doi:10.1016/j.vetmic.2012.09.026
- 337 4. Magnino S, Haag-Wackernagel D, Geigenfeind I, Helmecke S, Dovc A, Prukner-Radovic E,
338 Residbegovic E, Ilieski V, Laroucau K, Donati M, Martinov S, Kaleta EF (2009) Chlamydial
339 infections in feral pigeons in Europe: Review of data and focus on public health implications.
340 Veterinary microbiology 135 (1-2):54-67. doi:10.1016/j.vetmic.2008.09.045
- 341 5. Geigenfeind I, Vanrompay D, Haag-Wackernagel D (2012) Prevalence of Chlamydia psittaci
342 in the feral pigeon population of Basel, Switzerland. Journal of medical microbiology 61 (Pt
343 2):261-265. doi:10.1099/jmm.0.034025-0
- 344 6. Reinhold P, Ostermann C, Liebler-Tenorio E, Berndt A, Vogel A, Lambertz J, Rothe M,
345 Ruttger A, Schubert E, Sachse K (2012) A bovine model of respiratory Chlamydia psittaci
346 infection: challenge dose titration. PloS one 7 (1):e30125. doi:10.1371/journal.pone.0030125
- 347 7. Ostermann C, Ruttger A, Schubert E, Schrodler W, Sachse K, Reinhold P (2013) Infection,
348 disease, and transmission dynamics in calves after experimental and natural challenge with a
349 Bovine Chlamydia psittaci isolate. PloS one 8 (5):e64066. doi:10.1371/journal.pone.0064066
- 350 8. Senn L, Greub G (2008) Local newspaper as a diagnostic aid for psittacosis: a case report.
351 Clinical infectious diseases : an official publication of the Infectious Diseases Society of
352 America 46 (12):1931-1932. doi:10.1086/588562
- 353 9. Asner SA, Jatton K, Kyprianidou S, Nowak AM, Greub G (2014) Chlamydia pneumoniae:
354 possible association with asthma in children. Clinical infectious diseases : an official publication
355 of the Infectious Diseases Society of America 58 (8):1198-1199. doi:10.1093/cid/ciu034
- 356 10. Greub G, Sahli R, Brouillet R, Jatton K (2016) Ten years of R&D and full automation in
357 molecular diagnosis. Future microbiology 11:403-425. doi:10.2217/fmb.15.152
- 358 11. Welti M, Jatton K, Altwegg M, Sahli R, Wenger A, Bille J (2003) Development of a
359 multiplex real-time quantitative PCR assay to detect Chlamydia pneumoniae, Legionella
360 pneumophila and Mycoplasma pneumoniae in respiratory tract secretions. Diagnostic
361 microbiology and infectious disease 45 (2):85-95

- 362 12. Opota O, Jaton K, Branley J, Vanrompay D, Erard V, Borel N, Longbottom D, Greub G
363 (2015) Improving the molecular diagnosis of Chlamydia psittaci and Chlamydia abortus
364 infection with a species-specific duplex real-time PCR. Journal of Medical Microbiology.
365 doi:doi:10.1099/jmm.0.000139
- 366 13. Jaton K, Brouillet R (2011) Full automation of nucleic acid extraction with the dual starlet
367 (hamilton)-magnapure 96 (roche) system: A preliminary experience. ECCMID 2011, Milano
368
- 369

370 **Tables**

371

Table 1: Primers and probes

Pathogen	Oligonucleotide name	Target gene	Sequence (5'-3')	Modification, fluorochrome	Amplicon length	Final Concentration (µM)
<i>C. pneumoniae</i>	CPTM1	<i>pst1</i> gene	CATGGTGTTCATTGCGCCAAGT	-		0.2
	CPTM2	<i>pst1</i> gene	CGTGTCGTCCAGCCATTT TA	-		0.2
	CP	<i>pst1</i> gene	TCTACGTTGCCTCTAAGAGAAAACTTCAAGTTGGA	3'-VIC, 5'-TAMRA	82	0.1
<i>C. psittaci</i> and <i>C. abortus</i>	CPSI_F	16S-23S operon	AAGGAGAGAGGCGCCCAA	-		0.35
	CPSI_R_LNA	16S-23S operon	CAA[C]CTAGTCAAACCGTCCTAA	LNA		0.35
	CPSI_P_MGB	16S-23S operon	ACTGGGATGAAGTCGTAAC	FAM, DQ	133	0.2
<i>C. psittaci</i>	CPSI_00F	CDS CPSI_0607	AGCATTAGCCAGCGCTTTAGA	-		0.35
	CPSI_00R_147C/G	CDS CPSI_0607	TCTCTGAGCAAAAAC/GACTGCGT	-		0.35
	CPSI_00P_MGB	CDS CPSI_0607	ACAAAGACCTGGCGAGTA	VIC, DQ	118	0.2

LNA = Locked nucleic acid, MGB = Minor groove binder, FAM = 6-carboxy-fluorescein, VIC = TaqMan VIC reporter dye, DQ = Dark quencher, BHQ = Black hole quencher

372

373

Table 2. Clinical specimens and pathogens generally used for the diagnosis of *Chlamydia* infections

	Sample type	<i>C. pneumoniae</i>	<i>C. psittaci</i>	<i>C. trachomatis</i>	<i>C. abortus</i>
Oral	Mouth swab			x	
Respiratory specimen	Nasal swab	x	x		
Respiratory specimen	Nasopharyngeal secretions	x	x		
Respiratory specimen	Sputum	x	x		
Respiratory specimen	Bronchial secretion	x	x		
Respiratory specimen	Bronchoalveolar lavage	x	x		
Urogenital specimen	Uterus and urethral smear			x	x
Urogenital specimen	Urethral swab			x	
Urogenital specimen	Prostate biopsies			x	
Urogenital specimen	Fragment of placenta			x	x
Anal	Anal swab			x	
Osteo-articular	Joint fluid			x	
Osteo-articular	Prosthetic fragment			x	
Vascular	Drain fluid from aortic valve				
Animal specimen	Bird spleen		x		
Animal specimen	Birds choanal or cloacal swabs		x		
Animal specimen	Fragment of sheep placenta				x

Non-comprehensive list