Journal of Microbiological Methods 128 (2016) 20–23

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Note

Purification of infectious and non-infectious chlamydial particles using iodixanol for density gradient preparation

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ARTICLE INFO

Article history:
Received 6 April 2016
Received in revised form 27 June 2016
Accepted 28 June 2016
Available online 1 July 2016

Keywords:
Iodixanol
Visipaque®
Optiprep™
Intracellular pathogens
Preparative ultracentrifugation

ABSTRACT

Chlamydiae are obligate intracellular bacteria with two distinct morphological stages, the infectious elementary bodies (EBs) and non-infectious reticulate bodies (RBs). Here we describe a rapid and straightforward protocol for the purification of EBs and RBs involving special density gradients. It has been successfully applied to three chlamydial species.

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Chlamydiae are obligate intracellular Gram-negative bacteria with a noticeable impact on human and animal health (Longbottom and Coulter, 2003). Various chlamydial species like *Chlamydia* (*C.*) *abortus* and *C. psittaci* are animal pathogens that are evidentially zoonotic. Other *Chlamydiae* like *Waddlia* (*W.*) *chondrophila* were ascertained in animals and humans and exhibit a yet unknown zoonotic potential (de Barsy and Greub, 2013). All members of the phylum *Chlamydiae* share a characteristic biphasic developmental cycle where infectious, extra-cellular elementary bodies (EBs) and non-infectious reticulate bodies (RBs) alternate. EBs are about 300 nm, reveal a highly compacted nucleoid, structural rigidity and are responsible for dissemination of infection by invasion of susceptible cells. RBs are the lager (0.5–1 μm) metabolically active, replicative stage existing only inside the host cell (AbdelRahman and Belland, 2005). For many studies involving *Chlamydiae* it is crucial to apply highly purified EB and RB-fractions. Purification is achievable by different approaches fitting to certain demands. For example, Mukhopadhyay et al. (2004) used a prolonged infection period of 6 days and a huge multiplicity of infection (MOI) of 100 to achieve a high titer of EBs. Another objective was pursued by Friis (1972), who applied isopycnic gradient centrifugation to determine the precise density of chlamydial particles. Our approach should be appropriate for isolation of EBs and RBs within the first chlamydial propagation cycle and in sufficient amounts for gene expression analyses. Available protocols were inappropriate for the following reasons: usage of non-inert sucrose (Albrecht et al., 2011; Bose and Paul, 1982; Tamura and Higashi, 1963) and long implementation time due to isopycnic centrifugation (Friis, 1972; Howard et al., 1974) or consecutive ultracentrifugation procedures (Caldwell et al., 1981; Marques et al., 2010; Mukhopadhyay et al., 2004; Tamura et al., 1967; Wehrl et al., 2004). These deficiencies urged us to develop a novel, rapid purification approach yielding pure, native EBs and RBs.

A promising medium for gradient preparation was iodixanol a non-toxic, inert, non-ionic and isoosmotic medium widely used for organelle (Graham et al., 1994), virus (Lindenbach et al., 2005) or protein (Yee et al., 2008) purification. However, its suitability for purification of obligate intracellular bacteria remained unascertained. The chemical properties and the wide application of iodixanol allied with the deficiencies of other methods encouraged us to develop the present optimized protocol for EB and RB purification. Our demands were (I) avoiding high MOIs to mimic more natural infection conditions, (II) feasibility of the

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http://dx.doi.org/10.1016/j.mimet.2016.06.029
0167-7012/© 2016 Published by Elsevier B.V.

Table 1
Harvesting points, mean value of isolated RNA and infectious chlamydial particles in RB and EB fractions.

	Type	Harvest (hpi)	RNA (μg) ± SD	IFU ml^{-1} ± SD	EB:RB
C. abortus	RB	36	12.12 ± 3.29	$1.39 \times 10^6 \pm 1.1 \times 10^6$	206:1
	EB	48	20.3 ± 7.52	$2.86 \times 10^8 \pm 2.95 \times 10^{8*}$	
C. psittaci	RB	24	14.08 ± 3.2	$1.6 \times 10^6 \pm 6.1 \times 10^5$	146:1
	EB	38	31.7 ± 5.3	$2.4 \times 10^8 \pm 6.9 \times 10^{7**}$	
W. chondrophila	RB	18	11.89 ± 3.65	$2.18 \times 10^5 \pm 0.39 \times 10^5$	124:1
	EB	30	21.77 ± 5.76	$2.7 \times 10^7 \pm 8.83 \times 10^{6**}$	

Amount of RNA isolated and number of inclusion forming units (IFU) from purified RBs and EBs is represented as mean ± SD of independent replicates (n = 3). Statistics were conducted using Student's one-tailed *t*-test for independent samples.
* represents $P < 0.05$.
** represents $P < 0.01$.

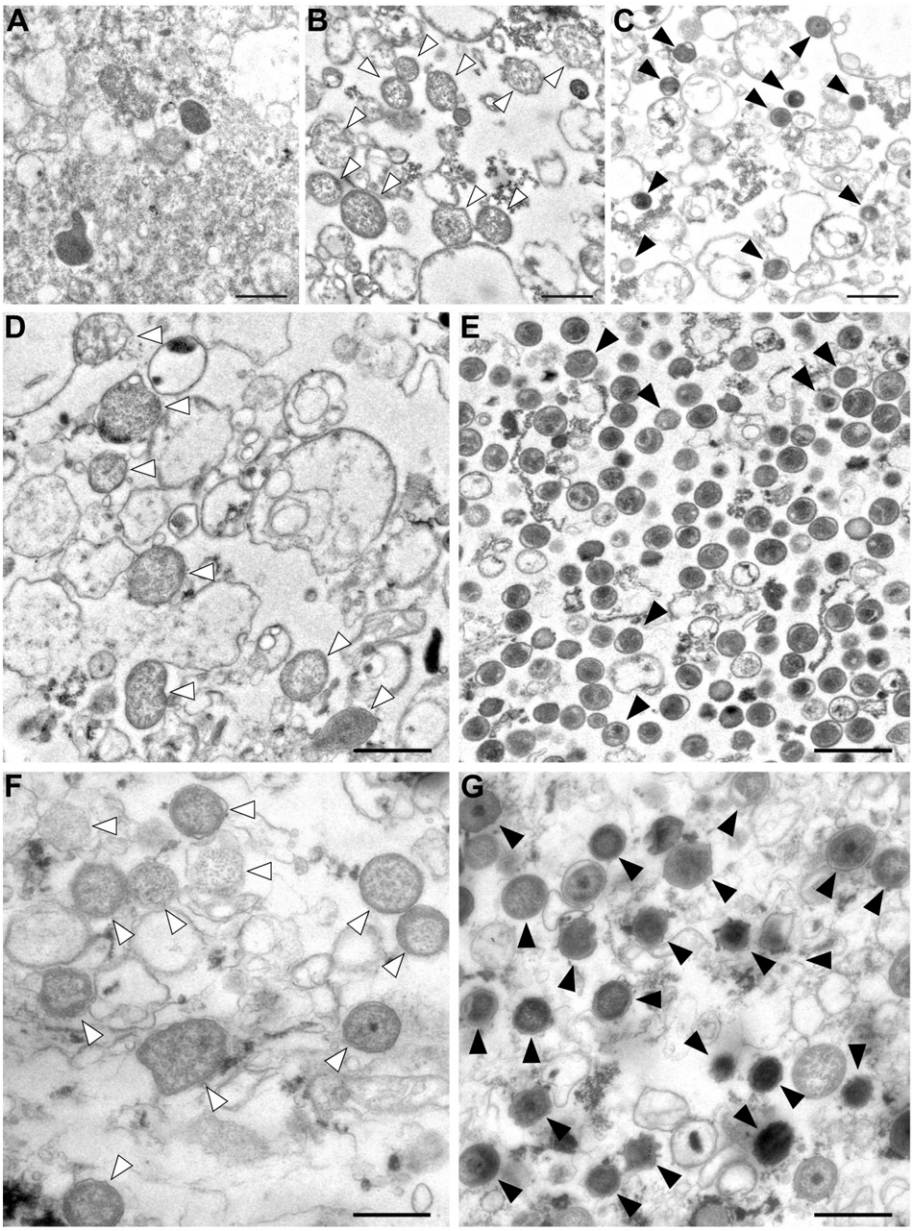

Fig. 1. RB and EB purification from various chlamydial species using iodixanol as density gradient medium. HEp-2 cells were infected with *C. abortus* (A, B and C), *C. psittaci* (D and E) and *W. chondrophila* (F and G) followed by purification of RBs and EBs. Representative transmission electron micrographs of crude extract (A), purified RBs (B, D and F) and EBs (C, E and G) are shown. In the crude extract, some chlamydial particles beside abundant granular and vesicular host cell debris can be seen. Host cell contaminants are depleted and chlamydial particles are concentrated upon the purification procedure. RBs appear as round to irregular particles with evenly dispersed granular material and a diameter of 0.5–1.0 μm (white arrowheads). EBs are smaller, round and contain powdery electron dense cell material (black arrowheads). RB fractions are virtually free of EB contaminations and *vice versa*. The scale bar of each image represents 1 μm.

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