1670. BME/CTVM2 cell line medium (Occidentia massiliensis)

Cultivation of *Occidentia massiliensis* has to be carried out inBME/CTVM2 cells. The cell line is NOT available from DSMZ but has to be obtained from: https://www.liverpool.ac.uk/infection-and-global-health/research/tick-cell-biobank/tick-cell-lines/

Cultivation of BME/CTVM2 cells for infection with *Occidentia massiliensis*

L-15 (Leibovitz) medium	70 ml
Tryptose phosphate broth	10 ml
Foetal calf serum	20 ml
L-glutamine (200 mM)	1 ml

All tick cell lines are incubated in an atmosphere of ordinary air, in sealed flasks or flat-sided tubes (Nunc, product no. 156758) in dry incubators. Medium changing is done once a week by removal and replacement of between 50% and 80% of the medium volume. Tick cells can survive for relatively long periods without attention – up to 3 weeks without a medium change -, and for several months without subculture if necessary. They prefer not to be subcultured too often!

Subculture is done when required, but not usually more often than fortnightly, as follows (method for 25cm2 flasks). On a medium change day, the spent medium from the culture to be passaged is put into the new flask(s) to condition them overnight. Next day the spent medium is discarded from the new flask(s), an appropriate volume of fresh medium (5ml for one new flask, 10ml for 2 new flasks etc) is added to the parent flask, any adherent cells are pipetted off, and the appropriate volume of cell suspension is transferred to the new flask(s), leaving 5ml in the parent flask. The parent flask is always retained, and can be used for subculture repeatedly over several months or years. Trypsin or EDTA are never used, as the tick cells do not like them.

Infection with *Occidentia massiliensis*:
From one 25 cm^2 flask of well-grown cells the medium is reduced to 5 ml, 3 ml of fresh medium are added and 1 ml of the *Occidentia* strain (quickly thawed from -80°C) is used to infect the cells. Incubate at 28°C with closed lid (without CO_2) and look for bacteria and destroyed cells after 7-10 days (using Gimenez staining after one week). To obtain more than 90% infected cells takes about 14 – 21 days.