The brain is protected from the entry of unwanted substances by means of the blood-brain barrier (BBB) formed by the brain microvasculature. This BBB is composed of non-fenestrated brain capillary endothelial cells (BCECs) with their intermingling tight junctions. The presence of the BBB is a huge obstacle for the treatment of central nervous system (CNS) diseases, as many potentially CNS active drugs are unable to reach their site of action within the brain. In vitro BBB models are, therefore, being developed to investigate the BBB permeability of a drug early in its development. The first part of the thesis involves the establishment and characterization of in vitro BBB models based on primary cells isolated from the rat brain. Co-culture and triple culture models with astrocytes and pericytes were found to be the superior to mono cultured BCECs with respect to many important BBB characteristics. In the second part of the thesis, the ability of turning BCECs into protein factories is investigated using a non-viral gene carrier. Transfection and protein synthesis of BCECs cultured with confined BBB properties were found to be feasible without disrupting the BBB properties, although it was not possible to demonstrate protein secretion of recombinant therapeutic polypeptides from BCECs. The third part of the thesis involves iron transport at BCECs, and the study of the transferrin receptor as a carrier for transport into the brain. The transferrin receptor is expressed by the BCECs and is involved in the uptake and transport of iron across the BBB. Expression of iron-related proteins was investigated at the BBB and it was possible to show expression of the essential iron transport protein; transferrin receptor, ferrireductases, divalent metal transporter 1 (DMT1), ferroportin and ferrooxidases, which was additionally confirmed in brain capillaries isolated from rats. In addition to astrocytes, pericytes were found to be a source of ferrooxidase activity within the brain, indicating that both of these cell types could be implicated in the control of iron release from BCECs and further transport into the brain. The exact pathway for iron transport by the transferrin receptor within BCECs was not fully elucidated, but two pathways seem possible.
To fulfill the requirements for the Ph.D. degree, Annette Burkhart Larsen has submitted the thesis: The blood-brain barrier in vitro using primary culture: Implications for studies of therapeutic gene expression and iron transport, to the Faculty Council of Medicine at Aalborg University.

The Faculty Council has appointed the following adjudication committee to evaluate the thesis and the associated lecture:

**Professor David Begley**  
King’s College London  
The United Kingdom

**Professor Thomas Andresen**  
Technical University of Denmark  
Denmark

**Chairman:**  
Associate Professor Cristian Pablo Pennisi  
Biomedicine Group, Aalborg University  
Denmark

**Moderator:**  
Professor Torben Moos  
Biomedicine Group, Aalborg University  
Denmark

The Ph.D. lecture is public and will take place on:

**Wednesday 17 December 2014 at 13:00**  
Aalborg University – Fredrik Bajers Vej 7 A4-106  
9220 Aalborg East

**Program for Ph.D. Lecture on**  
**Wednesday 17 December 2014**  
by

**Annette Burkhart Larsen**

The blood-brain barrier in vitro using primary culture: Implications for studies of therapeutic gene expression and iron transport

---

Chairman:  
Associate Professor Cristian Pablo Pennisi

Moderator:  
Professor Torben Moos

13.00 Opening by the Moderator

13.05 Ph.D. lecture by Annette Burkhart Larsen

13.50 Break

14.00 Questions and comments from the Committee
Questions and comments from the audience at the Moderator’s discretion

16.00 Conclusion of the session by the Moderator

After the session a reception will be arranged in the canteen area