Purpose: The microbiological and endothelial cell density (ECD) controls are carried out to assess corneal quality. Since 2005, the French Health Products Safety Agency (Afssaps) has been performing a routine external microbiological control on cornea media. Nowadays, an external ECD control on corneas might be developed. In this context, Afssaps organized a pilot study from 2008 to 2009 to validate the ECD control feasibility.

Methods: 5 volunteers Eye banks sent out of specifications corneas for ECD>2000 cells/mm², or positive anti-HBc, anti-HBs, anti-HBv, anti-CMV, anti-Toxo, anti-CMV. Six corneas from each Eye Bank were counted by banks using the routine methods and were counted by Afssaps by manual method and 2 analyzer techniques (border and centre methods). The counting results were compared.

Results: 68 corneas were sent to Afssaps in 29 parcels. Banks and Afssaps counts were made between 0 and 5 days. A manual method was used for the 68 corneas by eye banks and Afssaps. One bank (10 corneas) and Afssaps (61) used extra image analysis system. For manual counting: There was no significant difference between Afssaps (2166±710 cells/mm²) and eye banks (2266±298 cells/mm²). However, a significant difference was observed between manual counting in banks and analyzer method (+22% of cells with analyzer) and no significant difference between border and centre method with analyzer system. Conclusions: ECD control in Afssaps is feasible. An external quality control with all the French Eye Banks will be organized from 2011.

**RESULTS**

### COUNTING METHODS

- **Manual counting methods in Eye banks and for 10 of them with image analysis system in Afssaps**
- **68 corneas with manual counting methods in Eye banks and for 10 of them with image analysis system in Afssaps (border and centre method)**

### ECD CONTROL IN AFSSAPS IS FEASIBLE

- A significant difference between manual and image analysis counting was observed (+22% of cells with analyzer) and no significant difference between border and centre method with analyzer system.

### MATERIAL AND METHODS

#### CONTROLLED PRODUCTS

Five French Eye Banks (Lille, Lyon, Saint-Etienne, Marseille, Banque française des yeux) sent 68 out of specifications corneas to Afssaps.

#### SAMPLES TRANSPORT

The corneas underwent osmotic preparation in 0.9% NaCl to dilate the intercellular spaces and make endothelial cells contours visible before counting.

#### ENDOTHELIAL CELL DENSITY COUNTING METHOD

ECD were counted by the 5 Eye Banks and Afssaps using manual counting method. Afssaps and Saint-Etienne counting as well using analyzer counting methods. Border methods for the Saint-Etienne lab and border and centre methods for Afssaps.

#### MANUAL CELL COUNTING METHOD

Manual counting method was performed under a binocular direct light microscope through a reticle of known surface area and used a magnification x10 objective. The reticle was composed of a square grid divided into 20x10 identical square units, each 1 mm² and was positioned in one of the two eye pieces. The microscope and reticle were calibrated using a micrometric slide. This calibration permits to have a correcting factor. The initial cell count in the reticle image was multiplied by this factor to obtain ECD.

#### IMAGE ANALYZER COUNTING METHODS

Image Analyser (Sambacornea software) can determine ECD from light microscopic images function with two techniques:

- **Border method:**
  - It’s an individual cell detection based on the analysis of the contrast between the cell border and the intracellular space on images. The observer selected the endothelial areas to be examined. The contours of each EC in the selected area were automatically determined by Sambacornea. The observer manually corrected cell outlines that were incorrectly drawn by analyzer.

- **Centre method:**
  - It’s a manual or computerized assisted determination of the centre of each cell sometimes followed by the generation of hypothetical EC borders. For the both methods, the ECD, the mean CV of cell area and the number of cells with six neighbours [% hexagonality] were determined with a computer program. 2 images of each cornea were viewed at a time and a minimum of 300 cells were analyzed. The analyzer was calibrated with a standard certified micrometer.

**DISCUSSION/CONCLUSION**

The nonconformity of temperature during transport or during delivery doesn’t have effect on counts results. For manual counting, there was no significant difference between Afssaps (2178±536 cells/mm²) and Eye banks (1791±586 cells/mm²). The mean difference was 16%. For image analyzer: No differences as well (Afssaps: 2166±710 cells/mm², banks: 2266±298 cells/mm²). However, a significant difference was observed between manual counting in banks and analyzer method (+23% of cells with analyzer) and no significant difference between border and centre method with analyzer system. The period between Afssaps and Eye banks counts didn’t have influence on ECD results for manual or analyzer counting method.

### DISCUSION/CONCLUSION

The nonconformity of temperature during transport or during delivery doesn’t have effect on counts results. For manual counting, there was no significant difference between Afssaps (2178±536 cells/mm²) and Eye banks (1791±586 cells/mm²). The mean difference was 16%.

For image analyzer: There was no significant difference as well (Afssaps: 2166±710 cells/mm², banks: 2266±298 cells/mm²). However, a significant difference was observed between manual counting in banks and analyzer method in Afssaps (+23% of cells with analyzer).

### RESULTS

**ECD CONTROL IN AFSSAPS IS FEASIBLE**

- A significant difference between manual and image analysis counting was observed (+22% of cells with analyzer) and no significant difference between border and centre method with analyzer system.

**CONCLUSIONS**

ECD control in Afssaps is feasible. An external quality control with all the French Eye Banks will be organized from 2011.