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ABSTRACT

Purpose: The microbiological and endothelial cell density (ECD) controls are carried out to assess cornea grafts. 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 presence anti-Hbc + anti-Hbs. All the corneas were transported between +18 and +25°C. ECD 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 (1778 ± 536 cells/mm²) and Eye banks (1790 ± 568 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 in Afssaps (+17% of cells with analyzer). The period between Afssaps and banks counts didn't have influence on ECD results.

For Afssaps results, 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.

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:

- 41/68 for bad endothelial quality or Low Endothelial Cell Density (ECD);
- 27/68 for donor positive serology : presence Anti-HBC, Anti-HBs, EBV+, Toxo+, CMV+.

These samples were analysed, firstly, in the Eye Bank and then they were sent rapidly at ambient temperature quickly to Afssaps.

SAMPLES TRANSPORT

The samples were transported between +18°C and to 25°C to Afssaps by TSE Express Medical with a document indicating cornea sampling date, graft number, cornea bottling and endothelial cell density results in the Eye Bank.

The media packaging for the transport were constituted of three containers:

- first container : the bottle
- second container : plastic bag or polystyrene box
- third container : TSE box.

The 68 samples were sent in 29 distinct packages. 18/29 packages contained temperature sensor and the temperature curve was analysed. The temperature during the transport had to be between +18 to 25°C.

ENDOTHELIAL CELL DENSITY COUNTING METHOD

ECD were counted by the 5 Eye Banks and Afssaps using manual counting method.

Afssaps and Saint-Etienne were counting as well using analyzer counting methods. Border methods for the Saint-Etienne lab and border and centre methods for Afssaps.

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

MANUAL CELL COUNTING METHOD

Manual cell 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 10x10 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

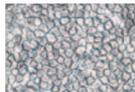
Image analyzer (Sambacornea software) can determine ECD from light microscopic images function with two techniques:

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 method, the ECD, the mean CV of cell area and the number of cells with six neighbours (% hexagonality) were determined with a computer program. 3 images of each cornea were viewed at a time and a minimum of 300 cells were analysed. 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 (1778 ± 536 cells/mm²) and Eye banks (1790 ± 568 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 border method in Afssaps (+17% of cells with analyzer).

The period between Afssaps and Eye banks counts didn't have influence on ECD results for manual or analyzer counting method.

Concerning Afssaps results:

A significant difference between manual and image analysis counting was observed (+22% of cells with analyzer for border method and +21% for centre method) and no significant difference between border and centre method with analyzer system.

RESULTS

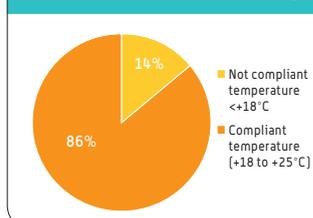
COUNTING METHODS

The corneas received were analysed:

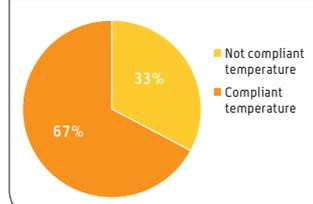
- 68 corneas with manual counting methods and image analysis system in Afssaps (border and centre method);
- 68 corneas with manual counting methods in Eye banks and for 10 of them with image analysis system (border method) in one eye bank.

TRANSPORT

Delivery temperature of 29 received packages

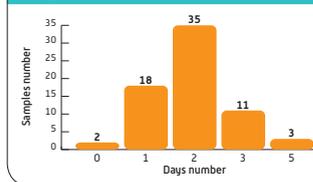


Temperature during transport for the 18 packages with temperature sensor



TIME BETWEEN AFSSAPS AND EYE BANKS COUNTS

Number of days between Afssaps and Eye Banks counts



Analysis of variance has been realized to show the influence of time between Afssaps and Eye banks counts:

- Number of days between Eye bank and Afssaps counts don't influence the ECD for manual counting (n=67);
- Number of days between Eye bank and Afssaps counts don't seem have influence on the ECD for analyzer counting (n=7).

STATISTICAL ANALYSIS OF COUNTS

	Corneas Number	Mean±SD (range, median)
Manual counting in Afssaps (method 1)	68	1778±536 (763-3328, 1781)
Border method with image analyzer Samba in Afssaps (method 2)	61	2166±710 (791-3570, 2134)
Centre method with image analyzer Samba in Afssaps (method 3)	61	2150±680 (772-3566, 2187)
Manual counting in Eye Banks (method 4)	68	1790±568 (400-3180, 1800)
Border method with image analyzer Samba in Eye Bank (method 5)	10	2266±298 (1981-2681, 2415)

The results are compared statistically with the paired samples procedure.

AFSSAPS COUNTS WITH DIFFERENT METHODS

	Border method with image analyzer Samba in Afssaps (Method 2)	Centre method with image analyzer Samba in Afssaps (Method 3)
Manual counting in Afssaps (Method 1)	Significant difference (+22% with method 2)	Significant difference (+21% with method 2)
Border method with image analyzer Samba in Afssaps (Method 2)		No significant difference

EYE BANKS AND AFSSAPS COUNTS COMPARISON

	Manual counting in Eye Banks (Method 4)	Border method with image analyzer Samba in Eye Bank (Method 5)
Manual counting in Afssaps (Method 1)	No significant difference	
Border method with image analyzer Samba in Afssaps (Method 2)	Significant difference (+17% with method 2)	No significant difference (Only 8 counts!)

In this pilot study on 68 corneas out of specification, we showed the feasibility of endothelial cell density quality control. The control could have been possible if the corneas had been sent to Afssaps quickly after the Eye bank counting (no influence showed until 5 days).

According to counting method used and the mean difference observed, a conclusion on the counting conformity could be applied like quality benchmark.

PROSPECTIVES

An external quality control on endothelial cell density in cornea with all the French Eye Bank will be organized from 2011.

ACKNOWLEDGEMENTS

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