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ABSTRACT

Purpose: The French Health Products Safety Agency (Afssaps) is the national competent authority for tissue control in France. A pilot study gathering all French eye banks was led from February to May 2005 with the view of validating the microbiological control feasibility and instituting a routine control, six series of controls were carried out from January 2007 to December 2008.

Methods: 112 parcels from 20 Eye Banks were transported to Afssaps at a range temperature from +2°C to +10°C. 84% of them contained a temperature sensor. 459 corneal media altogether (33% being sampling media, 65% storage media and 2% deswelling media) were analysed regarding their microbiological standpoint and transport conditions in parallel by Eye Banks and compared with Afssaps results.

80% of laboratories used manual or automated aerobic and anaerobic blood culture methods whereas 20% used seeded conventional methods. 80% of laboratories used a specific fungi media whereas Afssaps used blood culture bottles and Sabouraud medium. Moreover, the types of Bact/Alert seeded media depended on the volumes of media received.

Results: As a whole, the samples temperature was in accordance for 95% of them when received and 9% out of the 89 temperature curves analyzed came out to be non compliant during transport. The majority of the samples (325/459) were analyzed at Afssaps in a time schedule from 0 to 5 days after the banks' analyses. 3% of the results (13/459) were not compliant from the microbiological point of view and 2% (8/459) were contradictory between Afssaps and the Eye Banks. On the one hand, 7 samples out of 8 were positive according to Afssaps analyses and negative in the banks. On the other hand, 1 sample out of 8 were negative in Afssaps whereas positive in the banks.

Conclusion: 97% of the media controlled were negative from a bacteriological and fungal point of view. Positive controls were found in Afssaps although they were negative in the bank. This has led to either to destroy the corneas intended for the graft, or set up a biovigilance alert when the cornea had been grafted already and to carry out a follow-up of the receivers. This control makes sense when the banks send their media and when the control is carried out by Afssaps at the same period of time, so that the corneas can be destroyed before graft when necessary.

Since October 2005, then number of positive controls has decreased from 5% in 2005-2006 to 3% in 2007-2008. This decrease tends to follow the same trend as the bacteriological and fungal contamination rate relating to corneas which are declared positive each year by the French eye banks (10,4% in 2005-2006 and 8,6% in 2007-2008). The difference between the rate of contamination controls and the annual declarations is mainly due to the storage media for which there exists a factor of 2 between the rate found in controls and in the annual declarations. A media drawing lot sent to Afssaps for control would probably be necessary.

Moreover gathering the results of these quality controls in the longterm will enable to standardize the methods to work out reference factors for control.

MATERIAL AND METHODS

SAMPLES TRANSPORT

The samples were transported to Afssaps according to the regulations in force i.e. at a +4°C temperature by TSE (68% [76/112] of parcels by 13 banks on 20) or by others private companies (32% [36/112] of parcels by 7 banks on 20: Alpha transit, Cibex, Flash Europe, 300° services, Assistance Pharma Presto, Toxicolis) with a document indicating cornea bottling and microbiological control dates and time in the Eye Bank. The media packaging for transportation was constituted of three containers in most cases:

- First container: bottle or sample in sterile flask
- Second container: plastic bag with absorber or polystyrene box
- Third container: external container like TSE box, cool box or polystyrene box.

The 459 samples were sent in 112 distinct parcels. The temperature of the package was taken at delivery in Afssaps. 84% (94/112) contained temperature sensor and the temperature curve was analysed. The temperature during the transport had to remain between +2 and +10°C.

MICROBIOLOGICAL CONTROLS

In Eye Bank:

16/20 banks (80%) used automatic or manual, aerobic or anaerobic blood cultures bottles with an inoculum from 1 to 10 ml (for 5/16, inoculum ≤ 2.5 ml; 8/16, inoculum = 5 ml; and 3/16 inoculum = 10 ml) and an incubation time between 10 and 15 days (11/16 at 10 days). 4/20 banks (20%) seed conventional media (agar or broth), with an inoculum of 10 to 5 ml and an incubation time from 5 to 30 days. 16/20 (80%) banks used a specific fungi medium with an inoculum of 50 to 5 ml incubating from 5 to 30 days.

In Afssaps:

Volume of media received	Media seeding	Inoculum	Incubation
<15 ml	Bact Alert PF Bact Alert FN Sabouraud broth	4 ml maximum 10 ml maximum 1 ml	10 days 10 days 14 days
>15 ml	Bact Alert SA, SN, FA, FN, Sabouraud broth	10 ml maximum 1 ml	10 days 14 days

Germs are identified with identification systems like API® or ID32.

RESULTS

CONTROLLED PRODUCTS

459 samples of corneal media were sent to Afssaps from 20 Eye Banks in 2007 and 19 Eye Banks in 2008:

- 150 sampling media (126 Corneaprep II, 24 Corneamax),
- 298 storage media (Corneamax),
- 11 deswelling media (Corneajet).

All media received were analysed by Afssaps. 66% (303/459) of them were sent in their origin flask and 34% (156/459) in tube or other package aliquot.

These samples were firstly analysed in the Eye Banks and then either stored at +4°C before being sent to Afssaps or sent the same day.

- 150 milieux de prélèvement (126 Corneaprep II, 24 Corneamax)
- 298 milieux de conservation (Corneamax),
- 11 milieux de déturgence (Corneajet).

Ces échantillons ont été prélevés lors des contrôles effectués dans les banques puis ils ont été soit stockés à +4°C en attendant l'envoi à l'Afssaps, soit adressés le jour même.

TRANSPORT

DISCUSSION/CONCLUSION

5% of the 112 parcels were not in compliance with temperature when delivered. These differences firstly occurred because of the non-observance of the samples packing conditions (little or too much refrigerant).

The temperature during transport has been analyzed for 79% of parcels received (89/112). The results showed a non compliance for 9% of cases (8/89). 97% (446/459) of the media controlled were negative from a bacteriological and fungal point of view whereas 3% (13/459) were found positive. These controls were carried out with media samples stored up to 17 days after the bank analysis.

For 2% (n=8) results did not match between the banks and Afssaps although not linked to a transportation incident.

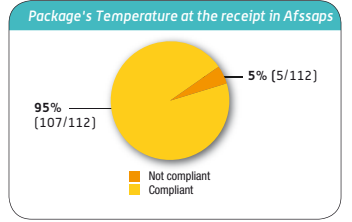
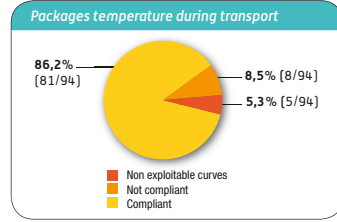
7 samples out of 459 (n° 2, 3, 4, 7, 8, 10 and 11) were found positive in Afssaps and negative in the banks (94% [7/13] of contaminated samples). The germs detected were known to be resistant to penicillin and streptomycin. This difference might be explained by the fact that to start with there might be a low level of contamination of samples which was not detectable by the techniques used by the banks. Afssaps analysis was performed after sample transportation and storage at +4°C which may allow germs to grow.

4 corneas coming from these positive media were grafted before Afssaps results were published. Therefore 4 biovigilance declarations were made and the recipient follow-up done. The 3 other corneas were destroyed before graft.

1 sample out of 459 negative (n° 14) were found negative in Afssaps and positive in the banks. This was explained by the occurrence of antibiotics in the media leading to a germ inactivation by a longer contact with the media. Enterococcus faecalis not detected can indeed be sensitive to penicillin. All the corneas resulting from the positive media found in the bank and in Afssaps (n° 1, 5, 6, 9, 12 and 13) were destroyed.

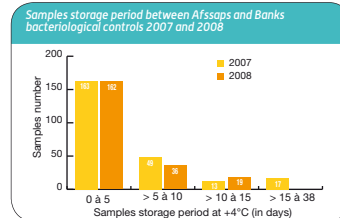
Since October 2005, then number of positive controls has decreased from 5% in 2005-2006 to 3% in 2007-2008. This decrease tends to follow the same trend as the bacteriological and fungal contamination rate relating to corneas which are declared positive each year by the French eye banks (10,4% in 2005-2006 and 8,6% in 2007-2008). The difference between the rate of contamination controls and the annual declarations is mainly due to the storage media for which there exists a factor of 2 between the rate found in controls and in the annual declarations.

Among the 112 parcels received, 94 contained a temperature sensor (84% of the cases) and 5 temperature curves during



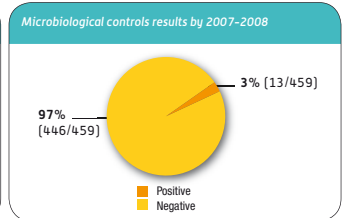
transportation were not transmitted. Among the 89 curves observed, 8 were not compliant with temperature during transport (9% of the cases).

SAMPLES STORAGE PERIOD BEFORE AFSSAPS ANALYSIS



The samples were analyzed from 0 to 38 days after the banks (distribution in the histogram below). The median is equal to 3 days.

MICROBIOLOGICAL CONTROLS RESULTS



3% of positive in 2007 (8/242) and 2% in 2008 (5/217).

Positivités					
N°	Media	Microbiological controls results of Afssaps	Microbiological controls results of the Eye Banks	Samples storage time at +4°C between Afssaps and Eye Banks microbiological controls (in days)	Aliquot or original flask
1	Corneaprep II Sampling medium	Positive (Micrococcus luteus et Sphingobacterium latipophilum)	Positive (Micrococcus species)	3h 30	Original flask
2	Corneamax Storage medium	Positive (Pseudomonas aeruginosa)	Negative	17	Aliquote en tube
3	Corneamax Storage medium	Positive (Wautersia paucula)	Negative	10	Original flask
4	Corneamax Storage medium	Positive (Wautersia paucula)	Negative	8	Original flask
5	Corneamax Storage medium	Positive (Candida guilliermondii)	Positive (Candida guilliermondii)	4	Aliquot
6	Corneaprep II Sampling medium	Positive (Sphingomonas paucimobilis)	Positive (Sphingomonas paucimobilis)	5	Original flask
7	Corneaprep II Sampling medium	Positive (Corynebacterium striatum ou amycolatum)	Negative	5	Original flask
8	Corneamax Storage medium	Positive (Ralstonia pickettii)	Negative	3	Original flask
9	Corneamax Sampling medium	Positive (Brevundimonas vesicularis)	Positive (Brevundimonas vesicularis)	7	Aliquot
10	Corneamax Sampling medium	Positive (Enterococcus faecium)	Negative	6	Aliquot
11	Corneaprep Sampling medium	Positive (Staphylococcus epidermidis)	Negative	1	Original flask
12	Corneaprep Sampling medium	Positive (Rhizobium radiobacter)	Positive (Rhizobium radiobacter)	8	Aliquot
13	Corneaprep Sampling medium	Positive (Stenotrophomonas maltophilia)	Positive (Stenotrophomonas maltophilia)	5	Aliquot

8 contaminated samples in 2007 (3,3%: 8/242) and 5 in 2008 (2,3%: 5/217); which make a total of 13/459 representing 3% in 2007-2008. 46% (6/13) are aliquot of original flask. 61% of the positive tests (8/13) were sampling media [rate of contamination of the received media of transport 5,3% (8/150)] and 38% (5/13) of the mediums of conservation [rate of contamination of the received storage media 1,7% (5/298)]. The positive media were analyzed in Afssaps from 0 to 17 days after the banks (54% of the cases (7/13) from 0 to 5 days afterwards).

Discordance

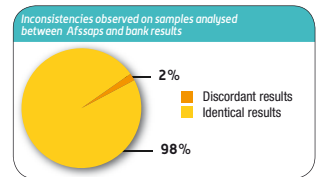
Out of 13 contaminated samples:

- 54% [7/13] have been found positive in Afssaps and negative in banks (board below).
- 46% [6/13] were matching.

One sample found negative in Afssaps and positive in the bank.

N°	Media	Microbiological controls results of Afssaps	Microbiological controls results of the Eye Banks	Samples storage time at +4°C between Afssaps and Eye Banks microbiological controls (in days)	Aliquot or original flask
14	Corneamax Sampling medium	Negative	Positive (Enterococcus faecalis)	2	Aliquot

8 bacteriological controls results on 459 were different between Afssaps and the banks.
 ■ 1,5% positive in Afssaps and negative in the banks.
 ■ 0,2% negative in Afssaps and positive in the banks.



A media drawing lot sent to Afssaps for control would probably be necessary.

To obtain relevant control and be able to react before graft in the case of a non compliance (fast destruction of the positive corneas): it is important to make blind controls and imperative to send the media samples to Afssaps within a maximum of 5 days after being analysed by the bank. An improvement has been observed in 2008 compared with 2007 where 33% of the media were not sent within the 5 days deadline (up to 38 days for some of them).

This control enables to observe the cornea preservation process realization and, especially in the case of a non compliance to discuss on the problems encountered, the bacteriological techniques and the media defrosting methods used with the view to improving the quality control by standardizing methods and techniques and setting up new national guidelines.

PERSPECTIVES

Perpetuating media controls and modifying the current procedure by carrying out a random control of graft number. Feasibility study of endothelial cell density controls realized from September 2008 to September 2009 to be able to set up the external control of the endothelial cell density.

ACKNOWLEDGEMENTS

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