

# BACTERIOLOGICAL AND FUNGAL CONTAMINATIONS OF CORNEAL ORGAN CULTURES MEDIA IN FRENCH EYE BANKS: 2008 RESULTS

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## ABSTRACT:

**Purpose:** The French Health Products Safety Agency (Afssaps) is the French national competent authority for the evaluation, inspection and laboratory control of tissues. Afssaps Laboratories and Controls Directorate (DLC) is in charge of the external quality control of tissues. Since 2005, the DLC has registered all micro-organisms in relation with bacterial or fungal contamination found in the corneal organ cultures media in the French Eye Banks. From 2005 till 2008, the comparison of different types of contaminations have been analysed.

**Methods:** Each Eye bank yearly sends its list of micro-organisms found in their corneal organ cultures media. The step in the process when the contamination appears, the microbiological tests performed, the germ detection time and the total number of controlled corneas are specified.

**Results:** In 2008, 19/20 French Eye Banks participated in the inventory. As a result, 8,3% (621/7493) of French cornea were destroyed because of a bacterial or fungal contamination. Moreover, 74% of the Eye Banks showed a cornea contamination rate below 10%. The number of controls, the procurement site number and the banks size activity do not affect the level of contamination. During the storage process, the majority of Eye Banks performed 3 microbiological controls.

The contaminations were detected by blood culture bottle methods (80% of banks) and 80% used a specific fungi media. The distribution of the germs was as follows: 78% of bacteria (26,2% genus *Staphylococcus*), 15% of yeasts (85,7% genus *Candida*), 2% of fungi (non-identified, *Fusarium*...). 0,3% of mycobacterium, 3% of mixture (bacteria, yeast and bacteria, yeast and fungi...). 2% of germs was not identified. Sampling media are more contaminated than the other media.

Since 2005, the national contamination rate has significantly decreased from 10,6% to 8,3% in 2008 (p<0,001). The germ distribution was equivalent to the 2005 value.

**Conclusions:** This inventory allowed Afssaps to have a better and extensive of micro-organisms responsible for cornea contaminations. It also enabled to define which germs had to be sent to the banks for new collaborative studies led by the DLC with the aim to validating or updating bacteriological reference frames. The contamination levels of the different Eye banks have decreased from 2005 till 2008. Two corrective actions were performed: staff mobilization to ensure a safer procurement in order to lower the rate of contamination and germs identification to know the origin of contamination.

In addition, since 2005, the contaminations with *Sphingomonas paucimobis* have been identified as coming from the bain-maries during the media defrosting process. It thus seems urgent to take measures making it possible to decrease these contaminations rates responsible for the loss of 300 corneas since 2005 approximately.

## BACTERIOLOGICAL AND FUNGAL CONTAMINATION DECLARED TO 2005 TO 2008

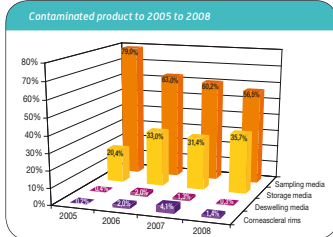
	2005	2006	2007	2008
Number of Eye banks	21	20*	20	19**
Contaminations observed	910	898	691	621
Corneas analyzed	8607	8749	7776	7493
% of corneas destroyed	10,6	10,3	8,9	8,3

\* Closure of one bank in 2006 and activity amalgamation in an other bank.  
\*\* Lack of data for one bank.

## CONTAMINATED PRODUCTS 2005-2008

	2005	2006	2007	2008
Sampling media	79% (718/910)	63% (569/898)	60,2% (416/691)**	56,5% (351/621)**
Storage media	20,4% (186/910)	33% (297/898)	31,4% (217/691)**	35,7% (222/621)**
Deswelling media	0,4% (4/910)	2% (15/898)	1,3% (9/691)	0,3% (2/621)
Corneal rins	0,2% (2/910)	2% (17/898)	4,1% (28/691)	1,4% (9/621)

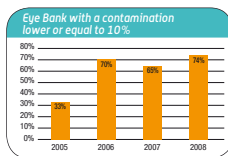
\* 3% in addition to contaminated media which were either sampling media or storage media (not specified by the bank).  
\*\* 4% in addition to contaminated media which were either sampling media or storage media (not specified by the bank).



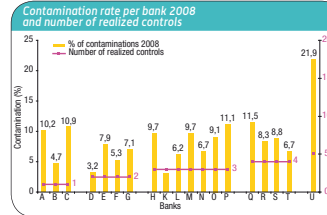
## CONTAMINATION RATE BY BANK

Bank	% of contaminations 2005	% of contaminations 2006	% of contaminations 2007	% of contaminations 2008	Number of controls	Control stage during the storage*
A	16,2	24,1	13,4	10,2	1	P - [C if change of medium colour]
B	7,8	6,2	7,9	4,7	1	[P if change of medium colour] - C
C	12,5	13,9	11,1	10,9	1	[P if change of medium colour] - C
D	1,4	5,9	1,9	3,2	2	P - C
E	10,1	7,6	7,7	7,9	2	P - [C if change of medium colour] - D
F	5,0	8,2	2,2	5,3	2	C - D
G	2,3	9,6	5,7	7,1	2	P - C
H	10,6	9,0	8,6	9,7	3	P - C ([J12] - C [J20])
I	3,4	5,5	4,9	/	3	P - C - D
J	11,0	Bank closure	Bank closure	Bank closure	3	P - C - D
K	6,6	5,5	1,0	3,0	3	P - C - D
L	15,0	13,6	12	6,2	3	P - C ([J10] - C (change of medium)
M	11,3	9,4	7,9	9,7	3	P - C ([J10] - C (change of medium)
N	14,6	11,0	10,3	6,7	3	P - C - D
O	12,2	4,1	11,7	7,1**+2	3	P - C - D
P	12,1	4,8	17,9	11,1	3	P - C - C - D
Q	10,7	11,5	3,4	11,5	4	P - C - D - CO
R	10,0	5,4	8,5	8,3	4	P - C - D - CO
S	10,5	20,0	23,7	8,8 + 2,8***	4	P - C - D - CO
T	13,4	8,7	8,2	6,7	4	P - C ([J10] - C (change of medium)
U	14,3	6,9	8	21,9	5	P - C ([J10] - C (change of medium) - D - CO

\* P: Sampling media, C: Storage media, D: Deswelling media, CO: Corneal rins.  
\*\* 0: Fungal contaminations no provided (about 2%).  
\*\*\* Bank S: it's the one bank to provide post graft contaminations (about 2,8%).

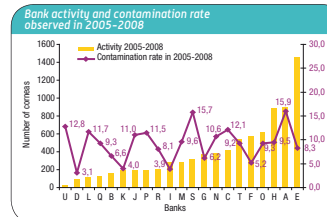


## INCIDENCE OF NUMBER OF REALIZED CONTROLS ON THE CONTAMINATION RATES PER BANK



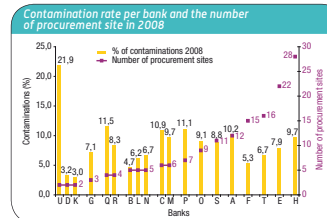
The median of controls number carried out by the banks is 3. No significant difference 5% ( $\alpha=0,09$ ) is highlighted between the contamination rates of the banks which carry out more than three controls and those which make less than three.

## INCIDENCE OF THE BANK ACTIVITY ON THE CONTAMINATION RATE TO 2005 TO 2008



The median of the bank activity is 287. No significant difference 5% ( $\alpha=0,2$ ) is highlighted between the banks which have an activity higher than 287 corneas per year and those which make less than 287.

## INCIDENCE OF NUMBER OF PROCUREMENT SITE ON CONTAMINATIONS RATES IN 2008



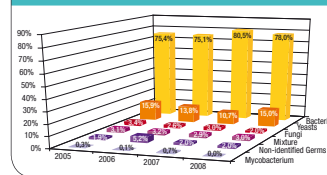
The median of procurement sites is 6. No significant difference 5% ( $\alpha=0,7$ ) is highlighted between the banks which have a procurement sites number higher than 6 and those which have less than 6.

## GERMS FOUND IN CONTAMINATIONS

	2005	2006	2007*	2008
Bacteria	75,4% (686/910)	75,1% (674/898)	80,5% (556/691)	78% (485/621)
Yeasts	15,9% (145/910)	13,8% (124/898)	10,7% (74/691)	15% (91/621)
Fungi	3,4% (31/910)	2,6% (23/898)	3% (21/691)	2% (15/621)
Mixture	3,1% (28/910)	3,2% (29/898)	2,9% (20/691)	3% (16/621)
Mycobacterium	0,2% (3/910)	0,1% (1/898)	0,7% (5/691)	/
Non-identified germs	1,9% (17/910)	5,2% (47/898)	2% (14/691)	2% (14/621)

\* A bank carried out in 2007 a Herpes detection and indicated it in contaminations list (0,1% of the contaminations).

## Distribution of germs found to 2005 to 2008



Bacteria: genus and species	2005 (75,4%)	2006 (75,1%)	2007 (80,5%)	2008 (78%)
<i>Staphylococcus</i>	34% (Staph coag negative 186/213; S.aureus 36/133)	30,9% (Staph coag negative 188/208; S.aureus 12/208)	22,3% (Staph coag negative 127/124; S.aureus 20/124)	24,2% (Staph coag negative 67/127; S.aureus 23/127; S.aureus 13/127)
<i>Pseudomonas</i>	20,4% (P.aeruginosa 82/410)	11,4% (P.aeruginosa 53/102; espèces non identifiées 12/65)	11,9% (P.aeruginosa 40/66; espèces non identifiées 12/65)	11,5% (P.aeruginosa 39/56; P.aeruginosa 12/56)
<i>Sphingomonas</i>	11,4% (S.paucimobis 62/78)	11,3% (S.paucimobis 57/76)	14,4% (S.paucimobis 67/90)	10,5% (S.paucimobis 48/51)
<i>Escherichia coli</i>	6,3%	6,8%	7,2%	7,5%
<i>Brevundimonas</i>	6% (B.vesicularis 38/11)	7% (B.vesicularis 14/17)	9,7% (B.vesicularis 52/54)	6,6% (B.vesicularis 23/32)
<i>Stenotrophomonas</i>	5,2% (S.mitotrophila 34/36)	5,8% (S.mitotrophila 39/39)	7,4% (S.mitotrophila 41/41)	4,3% (S.mitotrophila 19/21)
<i>Atalantia</i>	0,3%	0,1%	6,1% (P.aeruginosa 30/32)	4,3% (P.aeruginosa 18/18)
Non identified Gram-negative bacillus	2,3%	7,1%	2,5%	2,9%
<i>Agrobacterium</i>	1,9%	0,6%	0,4%	<1%
<i>Acinetobacter</i>	1,5% (A.baumannii 8/10)	0,3%	1,6% (A.baumannii 8/9)	2,7% (A.baumannii 11/13)
<i>Enterococcus faecalis</i>	1,4% (E.faecalis 5/10)	1,5% (E.faecalis 3/16)	2,9% (E.faecalis 17/16)	2,9% (Non identifié 4/11; E.faecalis 4/11)
<i>Achromobacter</i>	/	/	/	2,3%
<i>Methylobacterium</i>	/	/	/	2,1%
<i>Micrococcus</i>	0,3%	0,1%	1,1%	<1%
<i>Burkholderia cepacia</i>	1,2%	0,6%	0,4%	4,7%
<i>Proteus</i>	0,9%	1,5%	1,6%	<1%
<i>Propionibacterium</i>	0,3%	1,8%	2%	<1%
<i>Delftia</i>	0,1%	2,8% (D.acidovorans 17/17)	0,2%	/
Camissonia, Corynebacterium, Morganella, Serratia etc...	<1%	<1%	<1%	<1,5%

Yeasts: genus and species	2005 (15,9%)	2006 (13,8%)	2007 (10,7%)	2008 (15%)
<i>Candida</i>	63,4% (C.albicans 43/92; espèces non identifiées 22/192)	72,6% (C.albicans 42/90; C.glabrata 23/90)	87,8% (C.albicans 31/65; C.glabrata 12/65)	65,7% (C.albicans 42/78; C.glabrata 16/78)
<i>Levures non identifiées</i>	30,3%	25,8%	10,8%	8,8%
<i>Cryptococcus</i>	2,8% (C.tereus 4/1)	/	/	1,1%
<i>Rhodotorula</i>	1,4%	/	/	1,1%
<i>Saccharomyces</i>	1,4%	1,6%	1,4%	1,1%
<i>Trichosporon</i>	0,7%	/	/	1,1%
<i>Stachybotrys</i>	/	/	/	1,1%

Fungi: genus and species	2005 (3,4%)	2006 (2,6%)	2007 (3%)	2008 (2%)
Non-identified fungi	29%	47,8%	57,1%	53,3%
<i>Fusarium</i>	/	/	19%	6,7%
<i>Verticillium</i>	22,6%	/	/	/
<i>Aspergillus</i>	/	17,4%	14,3%	6,7%
<i>Penicillium</i>	12,9%	4,3%	4,8%	/
<i>Alternaria</i>	9,7%	8,7%	/	13,3%
<i>Acronium</i>	6,5%	/	4,8%	/
Autres genres	3,2% (Filletopis, Geotrichum, Aureobasidium, Absidia)	4,3% (Chaetomium, Engelmia, Rhinocladiella)	/	6,7% (Geotrichum, Mucor, Basidiomycetes)

## TECHNIQUES USED IN 2008

	April 2008	September 2008
Blood cultures bottles (manual or automatic)	Banks 81% (17/21)	80% (16/20)
Inoculum	1 to 10 ml	1 to 10 ml
Incubation	10 to 15 days	10 to 15 days
Conventional microbiological method (agar or broth)	Banks 19,1% (4/21)	20% (4/20)
Inoculum	10 µl to 5 ml	10 µl to 5 ml
Incubation	5 to 30 days	5 to 30 days
Fungi specific medium	76,2% (16/21)	80% (16/20)

(Data extracted from the collaborative studies scope of the tissue external quality control).

## DISCUSSION - CONCLUSION

The national contamination rate remains stable in 2008, with 8,3% versus 8,9% in 2007. The media which are mainly contaminated are always the sampling media.

Most of the banks carry out in average 3 bacteriological and fungal controls (9/20). Blood culture methods are used by 16 banks out of 20. The majority of the banks (16/20) add a fungi specific medium. The number of controls, the procurement site number and the banks size activity do not affect the level of contamination. No significant difference was highlighted.

As in previous years, the contaminations found in 2008 are mainly bacterial (approximately 78%). The most frequent germs to appear are the coagulase negative staphylococci, *Pseudomonas* and *Sphingomonas*.

To overcome these contaminations, several actions are to be undertaken: both to inform the procurement teams on the precautions to be taken to limit the contaminations at the procurement moment, and identify the germs found to seek for the cause of contaminations and carry out corrective actions.

Since 2005, the contaminations with *Sphingomonas paucimobis* have been identified as coming from the bain-maries during the media defrosting process. It seems urgent to take actions in order to decrease this contamination rate which led to the destruction of approximately 300 corneas since then. For instance: regularly changing the water, packing medium in a hermetic bag before defrosting, wiping the bottles before use, disinfecting the bottle when opening, could be some simple measurements to implement.

## ACKNOWLEDGEMENTS

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