

STERILITY MONITORING OF CAT STORED DONOR BLOOD

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Abstract. Contamination of donor blood is a permanent risk factor for blood transfusion. Using of non-sterile blood products can leads to severe complications and high health risks for recipient animals. Sterility research of canned blood, its components, canned bone marrow and blood transfusion products is required to detect possible contamination of aerobic and anaerobic microorganisms.

Factors of donor blood bacterial contamination may be blood collection systems, non-compliance of aseptic rules during blood collection, tightness violation of container, and others. As closed blood collection systems are not always available to veterinary practitioners, semi-closed systems or open (direct) blood collection are an alternative, which are at high risk for bacterial contamination of donor blood when used carelessly.

In our research, 12 canned cat donor blood samples were analyzed in total. Samples were stored for 30 days at +2-6 °C. The donor animals were clinically healthy 12 cats. Blood was collected from the jugular vein by semi-closed systems in polymeric containers with CPDA anticoagulant.

The bacterial culture method is considered as the "gold standard" for assessing the presence of blood contamination in most blood transfusion centers. The tested blood samples were inoculated into thioglycolate and Sabouraud medium and incubated in a thermostat at 20-25 °C. The incubation period was 14 days. According to the results of bacteriological examination of donor blood samples after their storage – non-sterile samples were not detected.

Thereby, semi-closed blood collection systems are reliable and allow to obtain donor blood samples without losing its sterility in long-term storage.

Keywords: animals blood transfusion, cat donor blood, microbial contamination, donor blood sterility

Introduction

Recently, the increase in indications for veterinary blood transfusion and its routine use in the veterinary practice caused a rise in the demand for animal donors. A high level of blood safety must be guaranteed to perform this procedure (Marenzoni et al., 2018).

In the last decade cats blood transfusion in veterinary practice is significantly developed. According to preliminary estimates, cat transfusion using packed red blood cells (pRBC) is increased in 3 times comparing to whole blood from 15 to 47 % (Brugue et al., 2018).

Blood transfusion can be a life-saving treatment with a crucial impact on anesthetic and surgical possibilities or intensive care, but it can never be considered totally safe. The development of infectious diseases in recipient cats is an iatrogenic risk that must be minimized by the highest standards of clinical veterinary practice (Pennisi et al. 2015).

FeLV is an oncornavirus that causes a variety of neoplastic and nonneoplastic diseases in cats. Transmission of the virus occurs primarily through saliva, but the virus is present in the blood and can be transmitted by blood transfusion. Testing of donor cats for the FeLV antigen by ELISA is recommended, and all seropositive cats should be excluded from blood donation. The Feline Immunodeficiency virus (FIV) is a lentivirus transmitted by exposure to the virus in saliva or blood. Testing of donor cats for FIV-specific antibodies by ELISA is recommended, and all seropositive cats should be excluded (Wardrop, 2005).

Blood can be a good nutrient medium for microorganisms, so the risk of bacteria growth in any blood product after it has been donated is significant.

Microbial contamination of donor

blood, its components and blood transfusion drugs are the one of urgent problems of clinical transfusion in veterinary practice. The risk of microbial contamination is possible at all stages during production of blood transfusion drugs. So microbiological testing above products takes special attention, particularly, for sterility testing (Lyubich, 2014).

Sterility research of stored blood, its components, canned bone marrow and blood transfusion products is required to detect possible contamination of aerobic and anaerobic microorganisms.

The reason of this is the situation when many iatrogenic factors may lead to bacterial contamination of blood products, which may cause local or system dangerous infection in a patient (Kessler et al., 2010). That happens despite the use of aseptic techniques during collecting and storage of blood in humane and veterinary medicine.

The main causes of blood components bacterial contamination during its processing may be a violation of the tightness of the container packaging or incorrect method of dividing of the transfusion drugs into several doses (Ischenkova et al., 2015).

Contamination may also occur when donor blood is collected. As closed blood collection systems are not always available to veterinary practitioners, semi-closed systems or open (direct) blood collection are an alternative, which are at high risk for bacterial contamination of donor blood when used carelessly (Brugue et al., 2018).

There have been reported cases of bacterial contamination of collecting blood caused by infection with *S. marcescens*, using inappropriate concentrations of disinfectant solutions for treatment of the cat's skin area before blood collection (Kessler et al., 2010; Hohenhaus et al., 1997).

Analysis of recent researches and publications

In cats, blood cannot be collected through a closed system and, therefore, collection of donor blood requires a multi-step manipulation of syringes and other devices. It is crucial that each step of the procedure is performed under the strictest aseptic conditions and that bacterial contamination of blood bags is prevented, as bacterial endotoxins can cause an immediate febrile reaction or even fatal shock in the recipient cat (Pennisi et al., 2015).

Blood transfusion drugs are usually visually inspected before use. The bacterial contamination should be suspected if there were discoloration, hemolysis of the upper layers of erythrocyte mass or visible clots. When such signs are detected, a bacteriological examination of this blood drugs should be started to determine if contamination has occurred or not. The bacterial cultivation method is considered as the “gold standard” for assessing the presence of blood contamination in most blood transfusion centers (Miglio et al., 2016).

The process of bacterial culture growth is slow as microorganisms take time to develop and reach a large number of cells. For this reason, there is possible an alternative - PCR method, since its analytical sensitivity is higher and the time required to obtain the final result is much shorter (Gary et al., 2006).

However, the properties of contaminating microorganisms determine their ability to grow under storage conditions. Only the presence of bacteria in storage blood is less important than their ability to replicate, which causes serious septic complications in patients (Miglio et al., 2016).

It should be noted that during sterility testing of blood transfusion drugs a detection of microorganisms is directly

proportional to their number in the test sample and depends on the ability of these microorganisms to produce visible growth on culture medium. At a low level of contamination, the probability of microorganisms detecting is very low, even in the case of evenly microbial contamination. Therefore, the goal of the sterility testing is proving of viable microorganisms' absence in the blood sample with the highest possible accuracy. The main factors that determine the effectiveness of sterility testing are sample volume for analysis, culture technique, composition of culture media, time and temperature of incubation of cultures (Lyubich, 2014).

Purpose. The purpose of this research is quality control testing of canned cat donor blood for sterility after storage. And evaluation of reliability of semi-closed blood collection systems for cat donor blood storage for up to 30 days while sterility maintaining.

Materials and methods of research

The materials were donor whole blood from 12 cats 1 to 5 years aged. The donor animals were clinically healthy. ELISA express testing on feline viral immunodeficiency (FIV) and cat leukemia had shown negative results for all donors (Marenzoni et al., 2018).

Blood was collected from the jugular vein by semi-closed systems. Cats donor blood samples were harvested in polymeric containers with CPDA anticoagulant (sodium Citrate, sodium Phosphate, Dextrose, Adenin).

The volume of blood sampling was calculated at 12 ml per 1 kg of animal weight. This amount minimizes unwanted donor cat risks during blood collection (blood pressure and heart rate) and after donation (Helm, 2010).

Blood samples was collected by semi-closed systems with the following technique: We have filled a 60 ml syringe with anticoagulant. Connected it to a tee and a butterfly-catheter. Carefully placed the donor in the thoracic position and raised its head. Shaved wool on the neck area, which held where venopuncture will be performed. Disinfected the skin with 70 % ethyl alcohol.

When the jugular vein was filled with blood and its contours were visible under the skin, inserted a butterfly-catheter upward through the skin into the vessel (Fig. 1). Blood was drawn into a syringe with carefully created negative pressure. The syringe was gently shaken to prevent blood coagulation and microscopic clots formation. Blood samples was taken slowly to prevent the vein collapse or sticking of needle cut to a vessel wall.

After collecting the right amount of blood, removed the catheter. A sterile gauze swab was applied to the venopuncture area for several minutes to prevent bleeding and hematoma formation. Transferred the

blood from the syringe to the polymer container. Tied nodes on the container tubes to form three segments in 5-10 cm to allow for additional diagnostic researches. The polymer container was signed with next data: date of donation, donor information, blood type, storage term.

The collected blood samples were typed with the “*RapidVet-H Feline*” test. All samples were defined as “B” blood type (Thollot, 2019). Also was providing a morphology blood testing of each sample by automatic hematology analyzer *Mindray BC2800*, the main results are shown in Table 1.

According to the approved Recommendation “Guide to the preparation, use and quality assurance of blood components” of the European Committee on Blood Transfusion (Guide, 2013), cats whole blood samples were storage at +2-6 °C conditions during 30 days in a special refrigerator for blood products (Fig. 2) on educational and scientific laboratory “Animal Blood Bank” of National University of Life and Environmental Sciences of Ukraine.



Fig. 1. Blood sampling using a semi-closed system in a cat

1. The results of hematology analysis of donor cats ($M \pm m, n = 12$)

Erythrocytes, 1012/l	Hemoglobin, g/l	Leukocytes, 109/l	Hematocrit, %
$8,9 \pm 1,8$	$125,6 \pm 5,9$	$12,2 \pm 2,7$	$39,2 \pm 5,8$

Bacteriological examination for the sterility of canned blood samples was performed according to the approved Instruction of the Ministry of Health of Ukraine (Instruction, 1999) in laboratory conditions on the Department of Microbiology, Virology and Biotechnology of NULES of Ukraine.

For testing we used sterile pipettes, closed with cotton plugs, rubber pears, sterilized tools, which were in a container with 96 % ethanol during manipulation.

Polymer containers with blood were checked for leaks in the lab pre-box, then wiped them 70 % ethanol. Before inoculation, we clamped the container tube above the node and cut the tube between the node and the clamp. The cut end of the tube was quickly passed through the flame, and a pipette was introduced into it. Reducing the pressure to the clamp, we pressed the container and piped at least 2 ml of sample.

Inoculation of each sample was made in a thick of culture medium without blowing a separate pipette but with a rubber pear and without previous flame processing. Used pipettes laid in a disinfectant solution.

The tested blood sample was inoculated with 1 ml into two tubes containing 20 ml of thioglycolate broth and one tube with 20 ml of Sabouraud medium. In parallel, two tubes with thioglycolate broth and one with Sabouraud medium were left untreated for controlling the sterility of the nutrient medium during the entire period of samples incubation. Cultures in thioglycolate broth and control tubes were incubated in a thermostat at 20-25 °C and at 30-35 °C, with Sabouraud medium at 20-25 °C (Fig. 3). The incubation period was 14 days for both culture media (Instruction, 1999).



Fig. 2. Refrigerator for storing blood transfusion products



Fig. 3. Thermostat of Microbiology laboratory

Accounting and interpretation of the sterility researching results. Cultures were viewed daily. The presence of the growth

of microorganisms in nutrient medium was assessed visually macroscopically (for the detection of turbidity, film, sediment, inclusions) and microscopically.

Results of the research and their discussion

Today in Ukraine there is no legal regulation in the field of animal blood donation. Also, there are a few little-known published experimental studies that would assess bacterial contamination of cat donor blood during the storage with using CPDA anticoagulant as a hemoconservative.

According to the Sterility Control Instruction (Instruction, 1999), the appropriate stage of control of canned donor blood is one of the main parameters for assessing its quality in the field of human health. With this in mind, the purpose of our work was to evaluate the suitability of semi-closed blood collection systems and risks of bacterial contamination of the cat donor blood by controlling its sterility after hypothermal storage.

2. The results of the blood samples examination for sterility

The animal	Contamination	The degree of contamination
1	—	—
2	—	—
3	—	—
4	—	—
5	—	—
6	—	—
7	—	—
8	—	—
9	—	—
10	—	—
11	—	—
12	—	—

Note: “—” - no contamination, “+” - presence contamination, “*” - low degree, “***” - middle degree, “****” - significant contamination

In total, we examined 12 canned cats blood samples which were used as donors in ESL “Animal Blood Bank” practice. Blood samples were tested after 30 days refrigeration storage at + 2-6 °C.

During the external examination of polymer containers with test samples, none of them showed signs of bacterial growth like a change of blood color from dark purple to red or green, a sign of hemolysis in upper layers of erythrocyte mass or visible clots.

The results of bacteriological research of blood samples using thioglycolate broth and Sabouraud medium showed that all 12 test samples with 30 days shelf-life were sterile (Table 2).

Conclusions and future perspectives of the study

The results of our research indicate that semi-closed blood collection systems with aseptic manipulations are reliable and allow to save the cats donor blood sterile up to 30 days.

The method of bacterial cultivation on thioglycolate broth and Sabouraud medium allows to evaluate the presence of preserved blood contamination after storage.

There is a need for further studies to assess hemolysis, erythrocyte counts and other parameters in canned cat donor blood in order to provide a high-quality product for cat blood transfusion.

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Егоров О. В., Малюк М. О., Козловська Г. В. (2020). КОНТРОЛЬ СТЕРИЛЬНОСТІ КОНСЕРВОВАНОЇ ДОНОРСЬКОЇ КРОВІ КІШОК. *Ukrainian Journal of Veterinary Sciences*, 11(1): 143–151, <https://doi.org/10.31548/ujvs2020.01.015>

Анотація. Контамінація донорської крові є перманентним фактором ризику за проведення гемотрансфузії. Використання нестерильних препаратів крові може призвести до тяжких ускладнень та значних ризиків для здоров'я тварин-реципієнтів. Дослідження на стерильність консервованої крові, як й її компонентів, препаратів, консервованого кісткового мозку та кровозамінників проводять з метою виявлення можливої контамінації аеробними та анаеробними мікроорганізмами.

Факторами бактеріального забруднення донорської крові можуть бути системи для забору крові, недотримання правил асептики під час забору крові, порушення герметичності контейнера тощо. Оскільки системи закритого способу забору крові не завжди доступні для ветеринарних практикуючих лікарів, альтернативою є напівзакриті або відкриті системи забору крові, які мають високий ризик щодо бактеріальної контамінації донорської крові за необережного її застосування.

Всього було досліджено 12 проб донорської консервованої крові кішок, які зберігались протягом 30 діб за температури + 2-6 °С. Тваринами-донорами були клінічно здорові 12 кішок, які мали негативні результати під час дослідження на вірусні імунodefіцит та лейкомію кішок. Кров відбирали з яремної вени напівзакритими системами. Зразки донорської крові кішок були заготовлені в полімерні контейнери з антикоагулянтном ЦФДА.

Метод бактеріального культивування вважається «золотим стандартом» для оцінки наявності контамінації крові у більшості центрів гемотрансфузії. Дослідні зразки крові засівали у тіюгліколевє середовище та середовище Сабуро. Посіви інкубували в термостаті за температури 20-25 °С. Термін інкубації становив 14 діб. За результатами бактеріологічного дослідження проб донорської крові після їх зберігання жодної нестерильної проби виявлено не було.

Таким чином, напівзакриті системи забору крові є надійними і дозволяють отримати донорську кров без втрати її стерильності за тривалого терміну зберігання.

Ключові слова: переливання крові тварин, донорська кров кішок, мікробна контамінація, стерильність донорської крові

Подано до друку 4 лютого 2020 року