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EVALUATION OF CLEAR D-TECT™ BIOLUMINESCENCE METHOD: A NEW CHEMICAL TOOL TO MONITOR IN 10 MINUTES THE MICROBIAL QUALITY OF HEMODIALYSIS WATER

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INTRODUCTION AND AIMS:

Microbial quality of dialysis water is monitored with 2 indicators: bacterial endotoxins levels and culture of bacteria. Plate counting is the standard method used to estimate the number of viable cells present in the water sample expecting that bacteria can give rise to CFU (Colony Forming Unit) under specific conditions of nutrient medium, temperature and time. Unfortunately there is no culture medium, temperature and incubation time suitable for all bacteria. For these reasons the numbers of CFU underestimate the microbial contamination. Furthermore an incubation time of 7 days is recommended. Safety management of microbial risk requires a reliable and fast method to detect a microbial contamination of dialysis water. A new kit Clear D-tect based on bioluminescence measurement of ATP (Adenosine TriPhosphate) is an alternative technique to rapidly detect viable bacterial cells in ultrapure water samples.

METHODS:

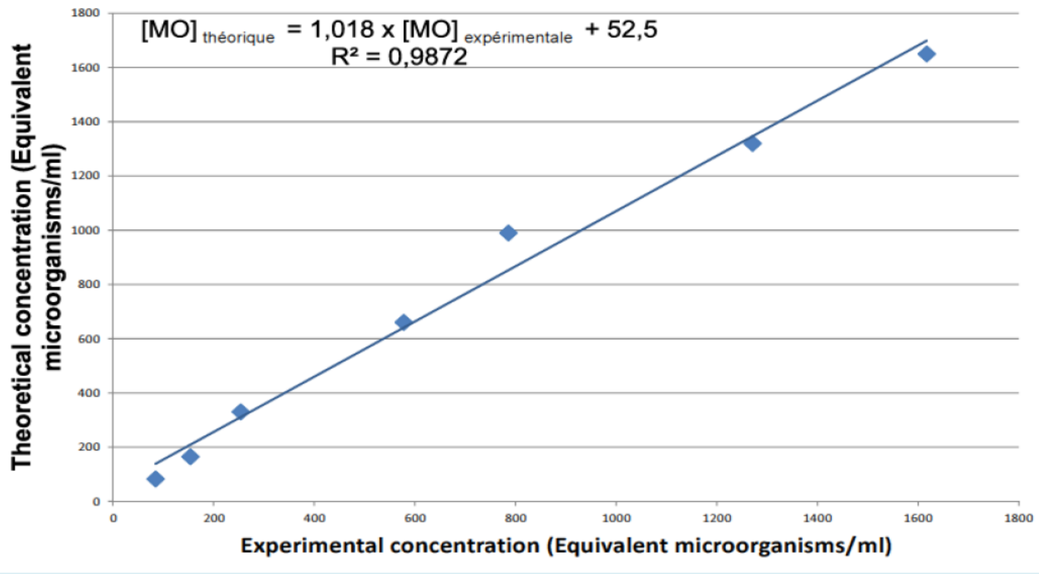
ATP is a molecule of energy storage in all living cells. Concentration of ATP in water samples is a good indicator of total living microbial flora contamination. Clear D-tect kit adapted for ATP measurement in ultrapure water samples was evaluated. The method is based on the filtration of water sample through a small single use filter to collect bacteria and eliminate extracellular ATP. From the viable cells collected, ATP is extracted and reacts with reagents of the kit. The intensity of light produced by the bioluminescence reaction measured in RLU (Relative Light Units) is calibrated using an ATP calibration reagent. Final result are expressed in pg of ATP/mL or Equivalent microorganisms/ml (1 Eq. microorganism = 1000pg cATP based on *E.coli* size bacteria ATP content). Performances of the method regarding the suitable volume of sample (50 or 100mL), 2 different concentrations of Luciferase enzyme and the linearity of the results were studied. A microbial mapping of a dialysis water treatment installation was performed.

Clear D-tect field test luminometer and kit

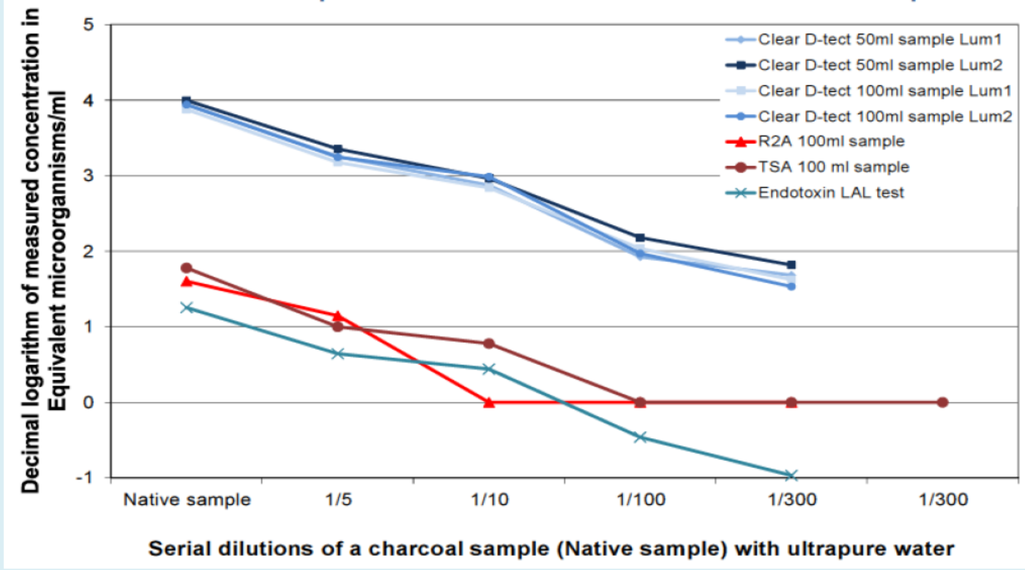
RESULTS 1: METHOD EVALUATION

The ATP measurements were not significantly different with 50 or 100 mL of water samples or regarding the 2 different concentrations of Luciferase enzyme tested. Very good linearity of results (R^2 = 0,9872) on serial dilution samples was obtained with R^2 of 0.9872 (Graph 1). Comparison of results between Clear D-tect, microbial count R2A reference method and Limulus test for endotoxins showed good correlation on serial dilution samples, with a better sensitivity for Clear D-tect and Limulus methods compared to the plate counts (Graph 2).

Graph 1: Linearity of Clear D-tect method



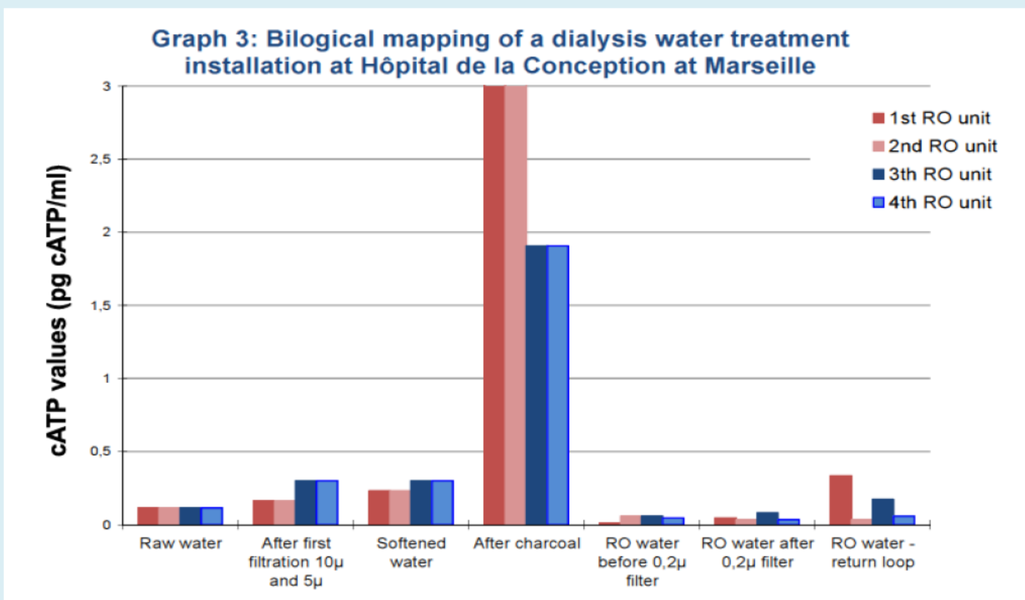
Graph 2: Comparison of different Clear D-tect protocols with R2A culture technique and endotoxin results on serial dilution samples



RESULTS 2: BIOLOGICAL MAPPING OF INSTALLATION

Microbial mapping of the water dialysis installation using ATP-metry showed comprehensive and logic evolution of microbial presence in the different sites of the water production installation (Graph 3), whereas microbial count is less adapted for measuring the flora in the pretreatment and in the reverse osmosis water.

Graph 3: Bilogical mapping of a dialysis water treatment installation at Hôpital de la Conception at Marseille



CONCLUSIONS:

Specific ATP-metry method developed for ultrapure water as Clear D-tect kit seems to be a good indicator of bacterial flora in dialysis installations. It is a convenient and reliable alternative to traditional microbial assays to monitor rapidly the quality of dialysis water. Good correlations between microbial contamination of hemodialysis water and cellular ATP levels have been obtained. Quantitative results for water bacteria contamination are obtained in less than 10 minutes versus 7 days with plate counting method. Further evaluations should be performed for determination of the threshold suitable for decision-making on dialysis fluid samples.

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