Comparison of NMKL no 66, 4th ed. 2003 and ISO 6888-1 and 2, 1st ed. 1999.

The methods that are described below are:

- NMKL no. 66, 4th ed., 2003. *Staphylococcus aureus*. Enumeration in foods.
- ISO 6888-1:1999 (E). Microbiology of food and animal feeding stuffs – horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) – Part 1: Technique using Baird-Parker medium.
- ISO 6888-2:1999 (E). Microbiology of food and animal feeding stuffs – horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) – Part 2: Technique using rabbit plasma fibrinogen agar medium.

Step	NMKL no 66, 4th ed., 2003	ISO 6888-1, 1st ed. 1999	ISO 6888-2, 1st ed. 1999
Principle	*S. aureus* is enumerated by surface plating on blood agar and/or Baird-Parker agar and/or Baird-Parker agar with Rabbit-Plasma-Fibrinogen (RPF). In this method *S. aureus* is defined as Gr+, catalase +, coagulase + cocci with typical colonies on blood agar or BP-agar, or Gr+, catalase + cocci that grow with typical colonies in BP-RPF.	Coagulase-positive Staphylococci are enumerated after inoculation of a specific quantity of the test sample onto a solid selective medium (BP) in duplicate. Coagulase positive staphylococci are defined as typical colonies on BP that are also cagulase positive. Applicable for food and animal feeding stuffs.	Coagulase positive Staphylococci are enumerated after inoculation of a specific quantity of the test sample onto a solid selective medium (BP-RPF) in duplicate. Coagulase positive staphylococci are defined as typical colonies on BP-RPF agar. Applicable for food and animal feeding stuffs.
Pre-treatment and diltution	Samples are mixed according to NMKL no 91, 2nd ed., 1988. 10 ml of liquid or 10 g of solid sample are mixed with 9x diluent and appropriate dilution series are made.	ISO 6887-1 and specific standards for the product concerned.	ISO 6887-1 and specific standards for the product concerned.
Plating, incubation and reading	0.1 ml of the appropriate dilutions of the test sample is surface inoculated onto blood agar and/or BP and/or BP-RPF and incubated for 48±4 hrs at 37.0±1.0°C. The plates are read after 24 and 48 hrs.	0.1 ml of the appropriate dilutions of the test sample is inoculated in duplicate onto the surface of BP-agar and incubate for 24±2 and a further 24±2 hrs at 35-37°C. The plates are checked after both 24 and 48 hrs.	1 ml of appropriate dilutions of the test sample is transferred in duplicate to empty Petri dishes and molten and temperated BP-RPF is poured into the dishes containing the sample. The plates are incubated at 35-37°C for 18-24 hrs and if necessary further 18-24 hrs. Typical colonies are counted.
Confirmation	A selection of typical and suspicious colonies is sub cultured onto non-selective agar and gram stained and checked for catalase activity and coagulase activity if the colonies are form BA or BP. If the colonies are from BP-RPF coagulase reaction takes place on the plate.	A selection of typical and atypical colony are checked in the coagulase test.	No further confirmation is needed as the coagulase test is included in the plates.

Expression of results	The number of *S. aureus* in the sample is calculated according to NMKL report no. 5, 2nd ed., 1994 and expressed as cfu/g or /ml sample.	The number of coagulase positive staphylococci is calculated and expressed as cfu/g or ml sample.	The number of coagulase positive staphylococci is calculated and expressed as cfu/g or ml sample.
Validated	Yes	Yes	Yes

Conclusion:

The methods described here may be considered as equal. The NMKL method includes the possibility of including Blood agar, but does not recommend using this as a single medium, but more like an extra plate. The ISO method states that the pour-plate method is used with BP-RPF agar thereby lowering the detection limit.

Both the NMKL method and ISO 6888-1and 2 have been validated in collaborative studies.