todylaboratories®

Microbiology Reagents CE

IVD

ST 16

Identification system for Staphylococci

REFERENCE CODE	
C53MR1069	(sp) Strip Plate – 60 tests

SUMMARY AND EXPLANATION

ST 16 kit is standardized identification system for common species Gram positive catalase positive microorganisms, especially of *Staphylococci*. Set is based on 16-17 miniaturized biochemical tests and software database. List of all microorganisms, for which is the kit determined, is placed at the end of leaflet.

PRINCIPLE

Kit is made of 16 wells strips of strippable microtitration plate in classic 96 wells format containing dehydrated substrates. Reconstitution of substrates runs by inoculation of bacterial suspension. During incubation occurs colour change in wells because of metabolic activity of microorganisms. Evaluation of results of tests is visual on base of colour scheme or by colour formulation described in leaflet. Results of identification are obtained from evaluation table or by help of evaluation software, which can be found at www.todylaboratories.com.

KIT CONTAINS

- 10 microtitration plates of ST 16
- 60 result forms
- 10 incubation packets
- 1 information leaflet

REQUIRED MATERIALS

Reagents:

- Unbuffered saline 3,5-5 ml
- Paraffin oil (Ref. C53MR1017)
- NIT reagent (Ref. C53MR1021)
- Zn (Ref. C53MR1028)

Optional:

- VP and VP reagent (Ref. C53MR1011 and C53MR1020)
- PYR and PYR reagent (Ref. C53MR1010 and C53MR1019)
- Identification software

Materials:

- Pipettes
- Tampons, loops, burner, tubes and other basic laboratory equipment.

SAMPLES

Isolate microorganisms, which have to be identified, from suitable unselective cultivation medium (for example blood agar, etc.) according to standard microbiological techniques. Make Gram colouring and microscope pure culture. Make catalase test. Confirmed isolates identify on ST 16.

RECCOMENDED PROCEDURE

Preparation of inoculum

- Open the tube with 2,5-5ml of unbuffered sterile saline.
- Take same well isolated colonies by inoculation loop from 18-24 hours old culture.
- Turbidity of homogenized suspension must be 3 McF.
  This suspension must be used immediately after preparation.

TIP: Eventually make proof of purity by braying of same loop or tampon, by which was suspension done.
This Petri dish can be used next day to make other additive tests!

Preparation of microtitration plate

- Prepare microtitration plate
- Mark strips with numbers of examining cultures.

Inoculation

- Inoculate by 0,1 ml of well homogenized suspension into each well of strip.
- Cover tests URE to ORN (wells H1 to F1) with 2 – 3 drops of paraffin oil.

Incubation

- Put the microtitration plate to packed PE packet, then bend end of packet under plate – this avoids dehumidifying of bacterial suspension.
- Incubate by common atmosphere at temperature 35 ± 2 °C for 18 - 24 hours.

TIP: For optimal incubation increase humidity in incubator for example by adding beak of water or incubate by incubator with managed humidity.

EVALUATION AND INTERPRETATION

We higlhly recommend to record the result of a NOV and COA!
Evaluate the tests after incubation with help of evaluating form, colour scheme, or by results of control batch.

Test bGA/NIT is bifunctional so after evaluating of primary reaction another result can be obtained from evaluated well of microtitration plate.

Make following optional bifunctional test
Well A1: bGA / NIT - after evaluation of primary reaction add 1-2 drops of NIT reagent and evaluate test NIT. Confirm negative test results with Zn dust. Color change after application of Zn dust means negative result. No color change confirm positive result!
Write down result of bifunctional tests to form for evaluating results or to evaluating software.

IDENTIFICATION

Result of identification can be obtained by:

- identification table
- identification software

Identification by identification table:

Compare results of tests and make identification by results of tests in identification table (page 4).

Identification by identification software:

Enter results of individual tests. When some of the test cannot be evaluated, it can be excluded. Software allows inputting additional tests and so it increases identification effectiveness.

microID software is for free in web based and web independed version.

QUALITY CONTROL

Quality of diagnostic kits is systematically controlled. Chemicals are bought only from certified companies and quality of these chemicals is confirmed by analytical certificate. The functionality of the kits is tested by collection of control strains, controlled and tested is also present of bacterial contamination. Kits are exposed to tests of higher temperatures and samples of each batch are saved for right advisement of later reclamations.

FOR THE NEED OF OWN PROOF FUNCTION, USE FOLLOWING RECOMMEND BACTERIAL STRAINS:

Enterococcus faecalis	CCM 4224 / ATCC 29212	H	G	F	E	D	C	B	A	A'
		URE	ARG	ORN	ESL	GLR	bGL	NAG	bGA	NIT
		-	-	-	+	-	+	+	-	-
		SUC	TRE	MAN	MLT	SOR	RAF	RIB	XYL	
		+	+	+	+	+	-	+	-	

Staphylococcu s aureus	CCM 3953 / ATCC 25923	H	G	F	E	D	C	B	A	A'
		URE	ARG	ORN	ESL	GLR	bGL	NAG	bGA	NIT
		+	-	-	-	-	+	-	-	+
		SUC	TRE	MAN	MLT	SOR	RAF	RIB	XYL	
		+	+	+	+	-	-	-	-	

s cohnii ssp.	CCM 4296/ ATCC 49331	H	G	F	E	D	C	B	A	A'
		URE	ARG	ORN	ESL	GLR	bGL	NAG	bGA	NIT
		+	-	-	-	+	-	-	+	+
		SUC	TRE	MAN	MLT	SOR	RAF	RIB	XYL	
		-	+	+	-	-	-	-	-	

pneumoniae ssp.	CCM 4 5852/ ATCC 13882	H	G	F	E	D	C	B	A	A'
		URE	ARG	ORN	ESL	GLR	bGL	NAG	bGA	NIT
		+	+	+	+	-	v	v	+	+
		SUC	TRE	MAN	MLT	SOR	RAF	RIB	XYL	
		+	+	-	+	+	+	+	+	

Profiles after 24 hour incubation on blood agar. Control strains serves to check functionality of individual tests only, not for proof control of identification.

CONSTRAINTS OF METHOD AND MOST OFTEN CAUSES OF WRONG IDENTIFICATION

- ST 16 kit is determined for identification of bacteria's named in this leaflet only.
- Only pure culture of microorganism can be used.
- Tests were not covered by paraffin oil.
- Contamination of wells by inoculum of next strip.
- Used culture is atypical batch.
- Some point of leaflet was not kept.

DETERMINATION CHARACTERISTICS

Tested were 62 collection strains and strains with clinical background, but veterinary strains mentioned in database were also tested:
- 91,9 % strains were identified correctly.
- 8,1 % strains were not identified

WARNINGS AND SAFETY PRECAUTIONS

- **For in vitro diagnostics use and microbial control**
- **For professional use only**
- Follow the instructions exactly!
- Used strips should be considered as potentially infectious and this must be respected when handling.
- Observe common safety measures according to the regulations of your country.
- Before use, check if the packaging is intact. Do not use damaged kit.

By interpretation of results anamnesis of patient, source of sample, morphology of colony, microscopic morphology of batch and if necessary, results of all overrun tests, mainly results of antibiogram have to be considered.

STORAGE CONDITIONS

ST 16 kits are delivered in multilayer packets on base of aluminium and organic polymers. Part of each packet is silica gel desiccant. Store kits at temperatures from +2 to +25°C. Expiration date is placed on each packaging.
Put unused opened microtitration strip in packed Al packet with original silica gel desiccant, close packet carefully and store it at laboratory temperature. Opened product can be stored two weeks or to date of expiration, in case when expiration date passes earlier.

WASTE LIQUIDATION

Work with material as with potentially infectious agens. Liquidate rubbish according to internal procedures and according to legislative of your country.

EVALUATING TABLE

Well – 1. line	SHORTCUT	NAME OF TEST	RESULTS	
			POSITIVE	NEGATIVE
H	URE	Urea	pink	yellow
G	ARG	Arginine	red-violet / red	yellow / yellow orange
F	ORN	Ornithine	red-violet / red	yellow / yellow orange
E	ESL	Esculine	brown	beige, light brown
D	GLR	b-glucuronidase	yellow	suspension turbidity
C	bGL	b-glucosidase	yellow	suspension turbidity
B	NAG	N – acetyl-glukosaminide	yellow	suspension turbidity
A	bGA	b-galaktosidase	yellow	colourless
A’	NIT	Nitrates	dark pink	suspension turbidity
Well – 2. line	**SHORTCUT**	**NAME OF TEST**	**POSITIVE**	**NEGATIVE**
H	SUC	Sacharose	yellow / grey yellow	violet / grey violet
G	TRE	Trehalose	yellow / grey yellow	violet / grey violet
F	MAN	Manitol	yellow / grey yellow	violet / grey violet
E	MLT	Maltose	yellow / grey yellow	violet / grey violet
D	SOR	Sorbitol	yellow / grey yellow	violet / grey violet
C	RAF	Raffinose	yellow / grey yellow	violet / grey violet
B	RIB	Ribose	yellow / grey yellow	violet / grey violet
A	XYL	Xylose	yellow / grey yellow	violet / grey violet

Identification table	URE	ARG	ORN	ESL	GLR	BGL	NAG	BGA	NIT	SUC	TRE	MAN	MLT	SOR	RAF	RIB	XYL
Dermatococcus nishinomiyaensis	v	-	-	-	-	-	-	-	v	-	-	-	-	-	-	-	-
Kocuria kristinae	-	-	-	(-)	-	+	-	(-)	-	+	+	-	+	(-)	-	-	-
Kocuria rosea	-	-	-	-	(-)	+	-	-	+	-	-	-	-	-	-	-	(-)
Kocuria varians	(+)	-	-	-	-	-	-	(-)	(+)	(-)	-	-	(-)	-	-	-	(-)
Kytococcus sedentarius	-	v	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Macrococcus bovicus	(-)	-	-	-	-	v	-	-	-	(-)	v	+	(+)	-	-	-	-
Macrococcus carouselicus	-	-	-	+	-	-	-	-	-	(+)	+	v	-	-	-	-	-
Macrococcus caseolyticus	-	-	-	-	-	-	-	-	+	v	+	-	+	-	-	(+)	-
Macrococcus equipercicus	(+)	-	-	(+)	-	-	-	-	-	-	v	+	(+)	-	-	-	-
Micrococcus sp.	(-)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Staphylococcus arlettae	-	-	-	v	+	-	-	v	-	+	+	+	+	-	+	+	+
Staphylococcus auricularis	-	v	-	-	-	-	-	(-)	+	v	+	-	(+)	-	-	-	-
Staphylococcus cap. ssp.ureolyticus	+	+	-	-	-	-	-	(-)	+	+	-	+	+	-	-	-	-
Staphylococcus capitis ssp. capitis	-	(+)	-	-	-	-	-	-	(+)	v	-	+	-	-	-	-	-
Staphylococcus caprae	+	+	-	-	-	-	-	-	+	(-)	+	+	+	-	-	-	-
Staphylococcus carnosus ssp. carnosus	-	+	-	-	-	-	v	+	+	-	(-)	(+)	-	+	-	-	-
Staphylococcus carnosus ssp. utilis	-	+	-	-	-	-	+	-	(+)	-	(+)	-	-	-	-	-	-
Staphylococcus cohnii ssp. urealyticum	+	-	-	(-)	+	v	v	v	(-)	-	+	+	(+)	(-)	-	-	-
Staphylococcus cohnii ssp. cohnii	-	-	-	-	(-)	-	(-)	-	-	-	+	v	(+)	(-)	-	-	-
Staphylococcus condimenti	+	+	-	-	-	-	+	+	+	v	+	+	-	+	-	-	-
Staphylococcus epidermidis	+	v	-	-	-	v	-	(-)	+	+	-	-	+	-	-	-	-
Staphylococcus eqourum	+	-	-	+	+	+	v	v	+	+	+	+	+	+	-	+	+
Staphylococcus felis	+	+	-	-	-	-	-	(+)	+	(+)	+	(+)	-	-	-	(-)	-
Staphylococcus gallinarum	+	-	-	+	(+)	+	+	v	+	+	+	+	+	(+)	+	+	+
Staphylococcus haemolyticus	-	+	-	-	(-)	v	+	(-)	+	+	+	v	+	(-)	-	(-)	-
Staphylococcus hominis ssp. novobiosepticus	+	-	-	-	-	-	-	-	+	+	-	-	+	-	-	(-)	-
Staphylococcus hominis ssp. hominis	+	(-)	-	-	-	-	(+)	-	+	+	(+)	(-)	+	-	-	-	-
Staphylococcus hyicus	v	+	-	-	(+)	v	+	-	+	+	+	-	-	-	-	+	-
Staphylococcus chromogenes	+	+	-	-	-	-	(-)	(-)	+	+	+	(-)	v	-	-	-	-
Staphylococcus klosii	v	-	-	+	v	(-)	-	v	-	-	+	+	+	-	(-)	+	(-)
Staphylococcus lugdunensis	v	-	+	-	-	(+)	+	(-)	+	+	+	-	+	(-)	-	-	-
Staphylococcus muscae	-	-	-	-	-	-	(-)	-	+	+	+	-	-	(-)	-	-	+

Staphylococcus pasteuri	+	(+)	-	-	+	+	-	-	(+)	+	+	(+)	(+)	+	-	-	-
Staphylococcus petrasii ssp.croceilyticus	+	+	-	-	+	v	-	-	(+)	+	+	-	+	-	-	-	-
Staphylococcus petrasii ssp.petrasii	+	+	-	-	-	+	-	-	+	+	+	-	+	-	-	-	-
Staphylococcus petrasii ssp. pragensis	-	-	-	-	v	v	-	-	+	+	+	-	+	-	-	-	-
Staphylococcus petrasii ssp. jettensis	-	+	-	-	v	v	-	-	+	+	+	v	+	-	-	-	-
Staphylococcus piscifermentas	+	+	-	+	-	+	v	-	+	v	+	(-)	v	(-)	-	(-)	-
Staphylococcus saprophyticus ssp. saprophyticus	+	-	-	-	-	(-)	(+)	(+)	-	+	+	+	+	-	-	-	-
Staphylococcus saprophyticus ssp. bovis	+	-	-	-	(-)	(-)	+	v	+	(+)	+	+	+	-	-	+	-
Staphylococcus sciuri ssp. carnaticus	-	-	-	+	-	+	-	-	+	+	+	+	+	(+)	-	+	+
Staphylococcus sciuri ssp. lentus	-	-	-	+	-	+	(+)	v	+	+	+	+	(+)	(+)	(+)	+	v
Staphylococcus sciuri ssp. rodentium	-	-	-	+	-	+	-	-	+	+	+	+	(+)	v	-	+	(-)
Staphylococcus sciuri ssp. sciuri	-	-	-	+	v	+	-	-	+	+	+	+	v	(+)	-	+	(-)
Staphylococcus schleiferi ssp. schleiferi	-	(+)	-	-	-	-	+	v	+	-	(+)	-	-	(-)	-	-	-
Staphylococcus simulans	+	+	-	-	v	-	+	+	+	+	+	(+)	(-)	(+)	-	v	-
Staphylococcus vitulinus	-	-	-	(+)	-	(+)	-	-	+	+	v	+	-	v	-	+	(+)
Staphylococcus warneri	+	v	-	-	(+)	+	-	(-)	(-)	+	+	v	+	-	-	v	-
Staphylococcus xylosus	+	-	-	(-)	+	+	0.9	(+)	(+)	+	+	+	+	-	-	v	+

Shortcuts: + = 90 – 99 %; (+) = 66 – 89 %; v = 34 – 65 %; (-) = 11 – 33 %; - = 1 – 10 %

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