CE

SBio

Antibiotic Susceptibility Tests

ANTIBACTERIAL/ANTIFUNGAL DISC AS PER CLSI (FORMERLY NCCLS) STANDARDS

Susceptibility Test Procedure

1. Prepare plates with Mueller Hinton Agar (AM1071/AM5071) for use in the Bauer-Kirby Method for rapidly growing aerobic organisms. For fastidious organisms such as Streptococci, the agar (AM1071/AM5071) is supplemented with 5% sterile, defibrinated blood. For *Haemophilus* spp. Haemophilus Test Medium and for *N. gonorrhoeae*, GC Agar Base (AM1046/AM5046) with 1% defined growth supplement are recommended respectively. The medium in the plates should be sterile and have a depth of about 4 mm.
2. Use pure cultures as inoculum. Select 3-4 similar colonies and transfer them into about 5 ml of suitable broth such as Tryptone Soya Broth (AM1092/AM5092). Incubate at 35-37^{0}C for 2-8 hours till light to moderate turbidity develops. Adjust the turbidity to yield a uniform suspension containing 10^5 – 10^6 cells/ml [i.e. 0.5 MCFarland standard (Cat. No.: 20701040)]. If the turbidity in the broth is sufficient, further incubation is not necessary.
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum (turbidity so adjusted, as to obtain confluent growth on the petri plate) and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60^0 angle between each streaking. Allow the inoculum to dry for 5 – 15 minutes with lid in place.
4. Apply the discs using aseptic technique. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
5. Incubate immediately at 35-37^{0}C and examine after 16-18 hours or later if necessary. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter.

Precaution

1. Accuracy of the test depends on the disc potency, proper inoculum, functional pretested medium plates (nature of medium and its depth), inoculation technique, incubation temperature and time etc.
2. To maintain the potency of discs, store the stock containers of discs in the freezer at -20^{0}C. The discs when required for use within a week , may be kept in refrigerator (below 8^{0}C) and the remainder should be kept with desiccant and tightly closed container caps in the freezer.
3. Remove the antimicrobial discs from refrigerator to room temperature 1-2 hours before use to avoid moisture condensation. Return unused discs to refrigerator immediately after applications.
4. Once a cartridge is opened it is recommended that it is stored for no more than one week.
5. While reading results:
   (a) Ignore swarming of *Proteus* species if zones of inhibition are clearly defined.
   (b) Measure the sulphonamide zones at the margin of heavy growth since Sulphonamide may not inhibit organisms for several generations and slight growth may appear within zones of inhibition.
   (c) Subculture, reidentify and retest any large colony growing within a clear zone of inhibition.
6. Control tests using known cultures should be included each time a sensitivity test is performed.
7. Antibacterial agents other than those listed in interpretative chart are in current use. Susceptibility tests employing these agents should be interpreted on the basis of presence or absence of a definite zone of inhibition and should be considered as only qualitative until the time interpretative zones have been established.

Note

1. Ampicillin disc is used for testing susceptibility to amoxycillin as well.
2. Cephalothin disc is used for testing susceptibility to Cephapirin, Cephaloridine, Cephalexin, Cefaclor, Cephoxitin, Cefazolin, Cephradine and Cephadroxil as well.
3. The Sulfisoxazole (Suphafurazole) disc can be used to represent any of the currently available sulfonamide preparations.
4. The category “Intermediate” should be reported. Infections with bacteria of intermediate susceptibility may be considered moderately susceptible and may respond to antimicrobial agents with a wide safe dosage range.
5. Since certain strains of *Providencia* spp. have been reported to give false susceptible results with Cefprozil discs, strains of this genus should not be tested & reported with this disc.
6. All *staphylococcal* isolates with zone diameters of 14 mm or less should be tested by an MIC (Minimal Inhibitory Concentration) method.
7. When testing Vancomycin against *Enterococci*, plates should be held a full 24 hrs & examined using transmitted light; the presence of a haze or any growth within the zone of inhibition indicates resistance.
8. Ofloxacin susceptible *S. pneumoniae* will also be susceptible to Levofloxacin.
9. Susceptibility & resistance to Azithromycin, Clarithromycin & Dirithromycin can be predicted by using Erythromycin for *Streptococci*.
10. The expiry date of the product is mentioned only on SBio carton and pouch.
11. SBio antimicrobial susceptibility test disc dispenser is provided inside the carton.

Zone Size Interpretative Chart

(Based on results obtained using Mueller Hinton Agar)

Product code	Antimicrobial Agent	Symbol	Diameter of zone of inhibition in mm											
			Disc content	Resistant mm or less	Intermediate mm	Sensitive mm or more	Quality Control, Limits							
							E.coli ATCC 25922	*S.aureus* ATCC 25923	*P.aeruginosa* ATCC 27853	*E.coli* ATCC 35218	*H.influenzae* ATCC 49247	*H.influenzae* ATCC 49766	*S.pneumoniae* ATCC 49619	*N.gonorrhoeae* ATCC 49226
900AK450	**Amikacin**	AK	10 mcg											
910AX450	**Ampicillin/Cloxacillin**	AX	10 mcg											
90AMX450 92AMX450 93AMX450	**Amoxycillin**	AMX	10 mcg 25 mcg 30 mcg											
95AMC450 91AMC450	**Amoxyclav** (**Amoxycillin/ Clavulanic acid)** When testing *Haemophilus* spp. and *Staphylococci* When testing *Enterobacteriaceae*	AMC	50/10 mcg 10 mcg											
910AP450 920AP450 930AP450	**Amphotericin-B**		100 units 20 mcg 50 mcg											
92AMP450 95AMP450	**Ampicillin** When testing *Enterobacteriaceae* When testing *Staphylococci* When testing *Haemophilus* spp. When testing *S.pneumoniae* When testing *Streptococcus* other than *S. pneumoniae* When testing *Enterococcus* spp.	AMP	2 mcg 25 mcg											
93AMS450	**Amoxycillin/Sulbactam**	AMS	30/15 mcg											
93AZM450	**Azithromycin** When testing *Haemophilus* spp.	AZM	30 mcg											
956AT450	**Aztreonam** When testing *Enterobacteriaceae* and *P. aeruginosa* When testing *Haemophilus* spp.	AT	50 mcg											
9100B450 9800B450 9040B450	**Bacitracin**	B	10 units 8 units 0.04 units											
93CPR450	**Cefadroxil (Cephadroxil)**	CPR	30 mcg											
930CN450	**Cefalexin (Cephalexin)**	CN	30 mcg											
95CPM450	**Cefepime**	CPM	50 mcg											
98CPT450 93CPT450	**Cefepime/Tazobactam**	CPT	80/10 mcg 30/10 mcg											
95CMC450	**Cefixime/ Clavulanic acid**	CMC	5/10 mcg											
93CFS450 91CFS450 95CFS450	**Cefoperazone/Sulbactam**	CFS	75/30 mcg 75/10 mcg 50/50 mcg											
97CST450	**Cefoperazone/Tazobactam**	CST	75/10 mcg											
91CTX450	**Cefotaxime (Cephotaxime)** When testing *Haemophilus* spp. When testing *S. pneumoniae* When testing *Streptococcus* other than *S. pneumoniae* When testing *N. gonorrhoeae*	CTX	10 mcg											
93CFP450	**Cefpirome**	CFP	30 mcg											
91CCL450	**Cefpodoxime/Clavulanic acid**	CCL	10/5 mcg											
93CAC450	**Ceftazidime/Clavulanic acid**	CAC	30/10 mcg											
98CAT450 93CAT450	**Ceftazidime/Tazobactam**	CAT	80/10 mcg 30/10 mcg											
91CTR450	**Ceftriaxone** When testing *Haemophilus* spp. When testing *S. pneumoniae* When testing *Streptococcus* other than *S. pneumoniae* When testing *N. gonorrhoeae*	CTR	10 mcg											
93CIS450	**Ceftriaxone/Sulbactam**	CIS	30/15 mcg											

Zone Size Interpretative Chart

(Based on results obtained using Mueller Hinton Agar)

Product code	Antimicrobial Agent	Symbol	Diameter of zone of inhibition in mm											
			Disc content	Resistant mm or less	Intermediate mm	Sensitive mm or more	Quality Control, Limits							
							E.coli ATCC 25922	*S.aureus* ATCC 25923	*P.aeruginosa* ATCC 27853	*E.coli* ATCC 35218	*H.influenzae* ATCC 49247	*H.influenzae* ATCC 49766	*S.pneumoniae* ATCC 49619	*N.gonorrhoeae* ATCC 49226
98CIT450 93CIT450	**Ceftriaxone /Tazobactam**	CIT	80/10 mcg 30/10 mcg											
9100C450 9500C450 9250C450	**Chloramphenicol** When testing *Haemophilus* spp. When testing *S. pneumoniae* When testing *Streptococcus* other than *S. pneumoniae*	C	10 mcg 50 mcg 25 mcg											
90CIP450 91CIP450 93CIP450	**Ciprofloxacin** When testing *Haemophilus* spp. When testing *N. gonorrhoeae*	CIP	1 mcg 10 mcg 30 mcg											
910CD450	**Clindamycin** When testing *Staphylococci* When testing *Streptococci*	CD	10 mcg											
910CC450	**Clotrimazole**	CC	10 mcg											
90COX450 95COX450 91COX450 93COX450	**Cloxacillin**	COX	1 mcg 5 mcg 10 mcg 30 mcg											
925CL450 950CL450	**Colistin (Methane Sulphonate)**	CL	25 mcg 50 mcg											
910DO450	**Doxycycline Hydrochloride**	DO	10 mcg											
9100E450 9500E450	**Erythromycin** When testing *Staphylococci, Entercoccus* spp. When testing *Streptococci*	E	10 mcg 5 mcg											
91FLC450	**Fluconazole**	FLC	10 mcg											
950FR450	**Furazolidone**	FR	50 mcg											
91GAT450 93GAT450	**Gatifloxacin** When testing *Enterobacteriaceae, Enterococcus* spp. When testing *Staphylococci*, When testing *Haemophilus* spp. When testing *Streptococci* When testing *N. gonorrhoeae*	GAT	10 mcg 30 mcg											
93GEN450 95GEN450	**Gentamicin**	GEN	30 mcg 50 mcg											
910IC450	**Imipenam/ Cilastin**	IC	10/10 mcg											
910IT450 930IT450	**Itraconazole**	IT	10 mcg 30 mcg											
9500K450	**Kanamycin**	K	5 mcg											
910KT450 930KT450 950KTC50	**Ketoconazole**	KT	10 mcg 30 mcg 50 mcg											
9200L450 9100L450 9150L450	**Lincomycin**	L	2 mcg 10 mcg 15 mcg											
91LOM450 93LOM450	**Lomefloxacin** When testing *N. gonorrhoeae* When testing *Haemophilus* spp.	LOM	15 mcg 30 mcg											
950MT450 940MT450	**Metronidazole**	MT	5mcg 4 mcg											
9300N450	**Neomycin**	N	30 mcg											
91NET450	**Netillin (Netilmicin Sulphate)**	NET	10 mcg											
91NIT450 92NIT450	**Nitrofurantoin**	NIT	100 mcg 200 mcg											
950NV450 930NV450	**Novobiocin**	NV	5 mcg 30 mcg											

Zone Size Interpretative Chart

(Based on results obtained using Mueller Hinton Agar)

Product code	Antimicrobial Agent	Symbol	Diameter of zone of inhibition in mm											
			Disc content	Resistant mm or less	Intermediate mm	Sensitive mm or more	Quality Control, Limits							
							E.coli ATCC 25922	*S.aureus* ATCC 25923	*P.aeruginosa* ATCC 27853	*E.coli* ATCC 35218	*H.influenzae* ATCC 49247	*H.influenzae* ATCC 49766	*S.pneumoniae* ATCC 49619	*N.gonorrhoeae* ATCC 49226
910NS450 950NS450	**Nystatin**	NS	100 units 50 mcg											
920OF450	**Ofloxacin** When testing *Haemophilus* spp. When testing *Streptococci.* When testing *N. gonorrhoeae* When testing *Staphylococci*	OF	2 mcg											
950OX450	**Oxacillin** When testing *Staphylococci* When testing coagulase negative *Staphylococci* When testing *S. pneumoniae*	OX	5 mcg											
9300O450	**Oxytetracycline**	O	30 mcg											
950PF450	**Pefloxacin**	PF	5 mcg											
9200P450	**Penicillin** When testing *Staphylococci* When testing *S. pneumoniae* When testing *Streptococcus* other than *S. pneumoniae* When testing *N. gonorrhoeae* When testing *Enterococcus*	P	2 units											
910PB450 950PB450	**Polymyxin-B**	PB	100 units 50 units											
91PRU450	**Prulifloxacin (Ulifloxacin)**	PRU	10 mcg											
92RIF450 91RIF450 93RIF450	**Rifampicin** When testing *E. coli,* When testing *Enterococcus* spp., *Staphylococci* & *Haemophilus* spp. When testing *S. pneumoniae*	RIF	2 mcg 15 mcg 30 mcg											
930RO450	**Roxithromycin**	RO	30 mcg											
903SR450 910SR450	**Spiramycin**	SR	30 mcg 100 mcg											
9250S450	**Streptomycin** When testing *Staphylococci*	S	25 mcg											
900SD450	**Sterile Discs**	SD												
910TE450	**Tetracycline** When testing *Haemophilus* spp. When testing *Streptococci* spp. When testing *N. gonorrhoeae*	TE	10 mcg											
93TOB450	**Tobramycin**	TOB	30 mcg											
910TR450 925TR450 930TR450	**Trimethoprim**	TR	10 mcg 25 mcg 30 mcg											
915TL450	**Tylosine**	TL												
950VA450 910VA450	**Vancomycin** When testing *Staphylococci* spp. When testing *Streptococci* spp. When testing *Enterococcus* spp.	VA												

Note:
1. For control limits of Gentamycin 120 mcg (AHLG00120005/AHLG00120010) use *E. faecalis* ATCC 29212, standard 16-23 mm (HLG).
2. For control limits of streptomycin 300 mcg (AHLS00300005/AHLS00300010) use *E. faecalis* ATCC 29212, standard 14-20 mm (HLS).

Δ : For *E. coli, S. aureus, P. aeruginosa* : Muller Hinton Agar. For Haemophilus spp: Haemophilus Test Medium; For *S. pneumoniae* : Muller Hinton Agar with 5% sheep blood;
For *N. Gonorrhoeae* : GC Agar Base with 1% defined growth supplement.

θ : Deterioration in Oxacillin disc content is best assessed with QC organism *S. aureus* ATCC 25923, with an acceptable zone diameter of 18-24 mm.

Antibiotic Susceptibility Tests

ANTIBACTERIAL/ANTIFUNGAL DISC AS PER NON CLSI (FORMERLY NCCLS) STANDARDS

Preparation of Inoculum

1. Inoculum is prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on Sabouraud Dextrose Agar (AM1087/AM5087) incubated at 35-37^{0}C. Colonies are suspended in 5 ml of sterile 0.85% Saline.
2. Vortex the resulting suspension and adjust the turbidity to yield 1 x 10^6 - 5 x 10^6 cells/ml [i.e. 0.5 MCFarland standard (Cat. No.: 20701040)].

Susceptibility Test Procedure

1. Prepare plates with Mueller Hinton Agar (AM1071/AM5071) +2% Glucose and 0.5 µg/ml Methylene Blue Dye (GMB) Medium for carrying out susceptibility of antifungal discs. The medium in the plates should be sterile and have a depth of about 4 mm.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum (turbidity so adjusted, as to obtain semi confluent growth on the petri plate) and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid, Streak the entire agar surface of the plate with the swab three times, turning the plate at 60^0 angle between each streaking. Allow the inoculum to dry for 5-15 minutes with lid in place.
3. Apply the discs using aspectic technique. Deposit the discs with centers at least 24 mm apart.
4. Invert the plates and place in an incubator set to 35-37^{0}C within 15 minutes after the discs are applied.
5. Examine each plate after 20-24 hours of incubation. If plate was satisfactorily streaked the resulting zones of inhibition will be uniformly circular and there will be a semi-confluent lawn of growth. Read at 48 hours only when insufficient growth is observed after 24 hours incubation.

Precautions:

1. Accuracy of the test depends on the disc potency, proper inoculum, functional pretested medium plates (nature of medium and its depth), inoculation technique, incubation temperature and time, etc.
2. To maintain the potency of discs, store the stock containers of discs in the freezer at -20^{0}C. The discs when required for use within a week, may be kept in refrigerator (below 8^{0}C) and the remainder should be kept with tightly closed container caps in the freezer.
3. Remove the antimicrobial discs from refrigerator to room temperature 1-2 hours before use to avoid moisture condensation. Return unused discs to refrigerator immediately after applications.
4. Measure the zone diameter to the nearest whole millimeter at the point at which there is prominent reduction in growth. Pinpoint micro colonies at the zone edge or large colonies within a zone are encountered frequently & should be ignored.

Zone Size Interpretative Chart for Antifungal Agent

(Based on results obtained on Mueller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye Medium)

Product Code	Antimicrobial agent	Symbol	Zone diameter, Nearest whole (mm)				Quality Control Limits					
			Disc content	Resistant mm or less	S- DD*	Sensitive mm or more	*C.albicans* ATCC 90028	*C.parapsilosis* ATCC 22019	*C.tropicalis* ATCC 750	*C. krusei* ATCC 6258	*C.albicans* ATCC 10231	*S.cerevisiae* ATCC 9763
AFLC00025005 AFLC00025010 AFLV00025005	Fluconazole	FLC	25 mcg	14	15-18	19	28-39	22-33	26-37	-	25-30	-
AVRC00001005 AVRC00001010 AVRV00001005	Voriconazloe	VRC	1 mcg	13	14-16	17	31-42	28-37	-	16-25	30-40	29-38

* : S - DD - Susceptible - Dose Dependent

Reference:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45:493.
2. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guidelines Vol.24 No. 15, May 2004 NCCLS document M44-A. For more details refer to this volume.
3. Performance Standards for Antimicrobial Disk susceptibility Tests, CLSI Vol. 29 No. 3, Jan. 2009. For more details refer to this volume.

Quality Control Limits for Antibiotics not as per CLSI
(Based on results obtained using Mueller Hinton Agar)

Antibiotics concentration of these products are as per customer requirements. These codes do not have concentration of antibiotics as per CLSI (Formerly NCCLS) standards.

Product Code	Antimicrobial Agent	Symbol	Disc Content	Diameter of zone of inhibition in mm		
				Quality Control Limits*		
				E.coli ATCC 25922	*S.aureus* ATCC 25923	*P.aeruginosa* ATCC 27853
930AK450	**Amikacin**	AK	30 mcg			
92AMC450	**Amoxycillin/Clavulanic acid**	AMC	20/10 mcg (30 mcg)			
91AMP450	**Ampicillin**	AMP	10 mcg			
910AS450	**Ampicillin/Sulbactam**	AS	10/10 mcg			
91AZM450	**Azithromycin**	AZM	15 mcg			
930AT450	**Aztreonam**	AT	30 mcg			
910CB450	**Carbenicillin**	CB	100 mcg			
930CF450	**Cefaclor**	CF	30 mcg			
930CZ450	**Cefazolin**	CZ	30 mcg			
95CDR450	**Cefdinir**	CDR	5 mcg			
93CPM450	**Cefepime**	CPM	30 mcg			
95CFM450	**Cefixime**	CFM	5 mcg			
97CPZ450	**Cefoperazone**	CFS	75 mcg			
93CTX450	**Cefotaxime (Cephotaxime)**	CTX	30 mcg			
91CPD450	**Cefpodoxime**	CCL	10 mcg			
93CFR450	**Cefprozil**	CFR	30 mcg			
93CAZ450	**Ceftazidime**	CAZ	30 mcg			
93CZX450	**Ceftizoxime**	CZX	30 mcg			
93CTR450	**Ceftriaxone**	CTR	30 mcg			
93CXM450	**Cefuroxime**	CXM	30 mcg			
93CEP450	**Cephalothin**	CEP	30 mcg			
9300C450	**Chloramphenicol**	C	30 mcg			
95CIP450	**Ciprofloxacin**	CIP	5 mcg			
91CLR450	**Clarithromycin**	CLR	15 mcg			
920CD450	**Clindamycin**	CD	2 mcg			
910CL450	**Colistin (Methane Sulphonate)**	CL	10 mcg			
91COT450	**Co-Trimoxazole (Trimethoprim/ Sulphamethoxazole)**	COT	1.25 / 23.75 mcg			
930DO450	**Doxycycline Hydrochloride**	DO	30 mcg			
91ETP450	**Ertapenem**	E	10 mcg			
9150E450	**Erythromycin**	E	15 mcg			
95FAR450	**Faropenem**	FAR	5 mcg			
92FLC450	**Fluconazole**	FLC	25 mcg			
95GAT450	**Gatifloxacin**	GAT	5 mcg			
95GEM450	**Gemifloxacin**	GEM	5 mcg			

Product Code	Antimicrobial Agent	Symbol	Disc Content	Diameter of zone of inhibition in mm		
				Quality Control Limits*		
				E.coli ATCC 25922	*S.aureus* ATCC 25923	*P.aeruginosa* ATCC 27853
91GEN450	**Gentamicin**	GEN	10 mcg			
91HLG450	**Gentamicin**	HLG	120 mcg			
91IPM450	**Imipenam**	IPM	10 mcg			
9300K450	**Kanamycin**	K	30 mcg			
950LE450	**Levofloxacin**	LE	5 mcg			
930LZ450	**Linezolid**	LZ	30 mcg			
91LOM450	**Lomefloxacin**	LOM	10 mcg			
91MRP450	**Meropenem**	MRP	10 mcg			
930MI450	**Minocycline**	MI	30 mcg			
950MO450	**Moxifloxacin**	MO	5 mcg			
930NA450	**Nalidixic Acid**	NA	30 mcg			
93NET450	**Netillin**	NET	30 mcg			
93NIT450	**Nitrofurantoin**	NIT	300 mcg			
910NX450	**Norfloxacin**	NX	10 mcg			
950OF450	**Ofloxacin**	OF	5 mcg			
910OX450	**Oxacillin**	OX	1 mcg			
9100P450	**Penicillin-G**	P	10 units			
910PI450	**Piperacillin**	PI	100 mcg			
91PIT450	**Piperacillin/Tazobactam**	PIT	100/10 mcg			
930PB450	**Polymyxin-B**	PB	300 units			
95PRU450	**Prulifloxacin (Ulifloxacin)**	PRU	5 mcg			
95RIF450	**Rifampicin**	RIF	5 mcg			
95SPX450	**Sparfloxacin**	SPX	5 mcg			
91SPT450	**Spectinomycin**	SPT	100 mcg			
9100S450	**Streptomycin**	S	10 mcg			
91HLS450	**Streptomycin**	HLS	300 mcg			
93TEI450	**Teicoplanin**	TEI	30 mcg			
930TE450	**Tetracycline**	TE	30 mcg			
975TI450	**Ticarcillin**	TI	75 mcg			
97TCC450	**Ticarcillin/Clavulanic acid**	TCC	75/10 mcg			
91TOB450	**Tobramycin**	TOB	10 mcg			
950TR450	**Trimethoprim**	TR	5 mcg			
930VA450	**Vancomycin**	VA	30 mcg			
91VRC450	**Voriconazole**	VA	1 mcg			

* : Expected diameter of zone of inhibition.

8°C -20°C Store at -20-8°C	Manufacturer	LOT Lot Number	Hygroscopic keep container tightly closed	REF Catalogue Number	Consult Instructions for use	Use by	IVD In vitro Diagnostic Medical Device	EC REP Authorised Representative	RO Received on	OO Opened on

Singapore Biosciences PTE Ltd.
11 Yishun Street 51, 304-23,The Criterion, Singapore 767971

EC REP
CMC Medical Devices & Drugs S.L., C/ Horacio Lengo No. 18, CP 29006, Malaga, Spain

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