3P® SMART Culture Media

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PERFORMANCES STUDY – CT 3P IR, TSA NEUT 3P IR, TSA 3P IR

DESCRIPTION

Traditional culture media is integral for any environmental monitoring program.[1,2] Whether it is used for microbial contamination monitoring or making product release decisions, choosing the right prepared culture media to deliver accurate results is essential.[3]

bioMérieux's irradiated 3P® SMART (Pharmaceutical Proven Performance) culture media is a premium product offering specifically designed for use in pharmaceutical cleanroom environments and have been validated for optimal performances particularly on environmental strains.

The following presents the performances of the 3P SMART culture media range during development, demonstrating to be the most comprehensive solution for an environmental monitoring (EM) program.

MATERIALS AND METHODS

bioMérieux Product Development

Product development at bioMérieux complies with ISO 13485 guidelines[4] and strict internal standards.

For each 3P SMART reference (Table 1), a minimum of 3 separate batches are manufactured for the purpose of Verification and Stability testing (shelf-life validation).

Verification testing is executed to ensure that the product can perform as intended.

Stability testing monitored in real-time, evaluates the 3 batches with applied thermal shocks to confirm that the product can consistently perform through expiration. Thermal shocks are applied to mimic storage conditions that could occur during shipping. Batches are then assessed at regular intervals for microbiological and physicochemical performance (i.e., growth promotion, neutralization capability when applicable, pH, and cosmetic aspect).

Table 1. 3P SMART References

Ref. #	Item Description	Plate Dish Format
420765	Irradiated Count-Tact 3P agar LOCKSURE (CT 3P IR)	Contact plate
423723	Irradiated Trypcase Soy 3P Agar with Neutralizers (TSA NEUT 3P IR)	90 mm settle plate
423725	Irradiated Trypcase Soy 3P Agar (TSA 3P IR)	90 mm settle plate

Microbiological Performance

During verification, growth promotion was evaluated with a total of 110 strains for CT 3P IR & TSA 3P IR and 94 microorganisms for TSA NEUT 3P IR that include pharmacopeia-required strains and cleanroom wild isolates sourced from an internal library collection.

Table 2. Number of strains tested per each microorganism category

Microorganism Type	CT 3P IR 110 Strains total	TSA NEUT 3P IR 94 Strains total	TSA 3P IR 110 Strains total
Molds	15	14	15
Yeasts	9	7	9
Gram Positive Cocci	22	21	21
Gram Positive Rods	19	18	19
Gram Negative Rods	38	33	40
Anaerobes	7	7	7

For stability, growth promotion was evaluated with the strains listed in Table 3 in BioBall® Multishot-550 format.

Table 3. Strains tested during Stability

Ref. #	Microorganism	BioBall Strain	Equivalent ATCC Strain
56011	*Aspergillus brasiliensis*	NCPF 2275	16404
56013	*Candida albicans*	NCPF 3179	10231
56012	*Bacillus subtilis*	NCTC 10400	6633
56016	*Escherichia coli*	NCTC 12923	8739
56017	*Pseudomonas aeruginosa*	NCTC 12924	9027
56019	*Staphylococcus aureus*	NCTC 10788	6538

List of Microorganisms:

Molds	Gram Positive Cocci	Gram Positive Rods	Gram Negative Rods		Yeast
Alternaria spp. 0411781	*A. viridans* 12950	*Aureobacterium spp* 0502715	*A. baumanii* ATCC 19606	*K. oxytoca* 104844	*C. albicans* ATCC 10231
A. brasiliensis ATCC 16404	*E. durans* ATCC19432	*B. cereus* ATCC 13824	*A. hydrophila* 101139	*K. pneumoniae* ATCC 13883	*C. glabrata* 300502
A. coryniformis ATCC 46373	*E. faecalis* ATCC 29212	*B. brevis* 0010703	*A. sobria* 111035	*L. adecarboxylata* 113532	*C. sake* 301604
A. fumigatus 0411768	*E. faecium* 12834	*B. coagulans* ATCC 9172	*A. cryaerophilus* 140160	*L. adecarboxylata* 113764	*C. neoformans* ATCC 32045
A. fumigatus 1401601	*E. gallinarum* 12838	*B. firmus* ATCC 29789	*B. vesicularis* 101556	*M. mesophylicum* 0306751	*G. candidum* 300575
A. terreus 0411763	*K. kristinae* 18527	*B. licheniformis* 200019	*B. cepacia* ATCC 25416	*P. vulgaris* 104972	*R. mucilaginosa* 305157
C. keratinophilum 0411774	*K. rhizophila* ATCC 9341	*B. megaterium* 200061	*B. gladioli* 110263	*P. stuarii* ATCC 43258	*S. cerevisiae* ATCC 9763
E. floccosum 0411793	*K. rosea* 8425	*B. pumilus* 2000111	*C.indologenes* ATCC 29897	*P. aeruginosa* ATCC 9027	*T. mucoides* 304040
P. lilacinus ATCC 90461	*Micrococcus* spp. 10085	*B. simplex* 202696	*C. luteola* 0311707	*P. aeruginosa* ATCC 14207	*Z. rouxi* 305693
P. aurantiogriseum ATCC 16025	*S. aureus* ATCC 6538	*B. subtilis* ATCC 6633	*Citrobacter braakii* 100772	*P. fluorescens* 106364	
P. commune 0909600	*S. aureus* ATCC 25923	*B. nesterukovii* 1401619	*Citrobacter freundii* ATCC 43864	*P. putida* 101109	
P. italicum ATCC 48114	*S. capitis* 2550	*B. polymyxa* ATCC 11330	*C. tertigena* ATCC 11330	*R. aquatilis* 109226	
R. pusillus 0411761	*S. epidermidis* 3052	*B. epidermidis* ATCC 35514	*D. acidovorans* 106523	*R. planticola* 104937	
S. brevicaulis 0411778	*S. epidermidis* ATCC 12228	*C. haemolyticum* 9602770	*E. aerogenes* ATCC 13048	*S. enterica* ATCC 19126	
T. mentagrophytes ATCC 9533	*S. haemolyticus* 3047	*C. striatum* ATCC BAA-1293	*E. cloacae* 105620	*S. gallinarum* 106305	
	S. haemolyticus ATCC 29970	*L. mesenteroides* ATCC 23386	*E. coli* ATCC 8739	*S. fonticola* 106356	
	S. hominis 8045	*L. fusiformis* 202043	*E. coli* ATCC 25922	*S. liquefaciens* ATCC 27592	
	S. pyogenes ATCC 19615	*P. lautus* 202122	*F. oryuhabitans* 0012704	*S. flexneri* ATCC 33948	
	S. sapnophyticus 3039	*V. paratortherticus* 202138	*H. alvei* ATCC 13337	*S. pascimobilis* 108706	
	S. warneri 12953			*S. maltophilia* 109782	
	S. warneri 12956				

Anaerobes
B. fragilis ATCC 25285
B. vulgatus ATCC 8482
C. perfringens ATCC 13124
C. sporogenes ATCC 11437
P. acnes ATCC 6919
P. granulosum ATCC 25564

Calibrated inoculum between 10 and 100 CFU from fresh cell suspension or BioBall format was prepared for each microorganism. Plates were inoculated in duplicate and incubated at 20-25°C or 30-35°C according to the growth requirements of the microorganism for an appropriate culture duration.

The recovery rates were calculated from a non-irradiated TSA control with an acceptance criteria between 50-200%.

Neutralization Capability

CT 3P IR and TSA NEUT 3P IR contain neutralizing components to inactivate any residual disinfectants present in the air, operator gloves, or surface to be tested.

These 3P products were challenged to evaluate their ability to neutralize commercially available disinfectants from different classification categories – four (4) for CT 3P IR and three (3) for TSA NEUT 3P IR. The recovery was compared to counts on non-irradiated TSA control (without disinfectant) with an acceptance criteria between 50-200%.

Table 4. Commercially available disinfectants tested per disinfectant type

Alcohol	Oxidizing Agents	Phenolic Compounds	Quaternary Ammonium Compounds
70% IPA	Clorox®	**Vesphene®***	**Decon Quat® 100**
	Spor-Klenz®*	Sporicidin®	Texwipe® Tex Q™ TX 650
			Coverage® Spray TB*

*Disinfectants tested only on TSA NEUT 3P IR
Disinfectants in bold tested during Stability

Neutralization was assessed against two groups of microorganisms: USP <61> required[5] and common wild isolates during verification (Table 5), and strains tested during stability are shown in Table 6.

Table 5. Microorganisms tested during Verification

Pharmacopeia Required	Supplemental Organisms (including wild isolates)
C. albicans ATCC 10231	*S. paucimobilis* 108706
A. brasiliensis ATCC 16404	*C. striatum* ATCC BAA-1293
P. aeruginosa ATCC 9027	*K. rhizophila* ATCC 9341
S. aureus ATCC 6538	*B. pumilus* 200111
B. subtilis ATCC 6633	*E. coli* ATCC 8739

Table 6. Microorganisms tested during Stability

Strain BioBall Multishot Format	Equivalent ATCC Strain
Bacillus subtilis NCTC 10400	ATCC 6633
Staphylococcus aureus NCTC 10788	ATCC 6538
Candida albicans NCPF 3179	ATCC 10231

Each disinfectant was prepared based on a pre-determined concentration that inhibits growth on standard TSA (without neutralizers) but are effectively neutralized by the compounds in the 3P media according to the microorganisms' sensitivity to each product. Disinfectant solutions of 200 µL for contact plates and 500 µL for settle plates were applied directly to the surface and allowed to dry prior to inoculating the designated microorganism.

Incubation conditions: up to 48 hours at 30-35°C for bacteria and up to 7 days at 20-25°C for yeast and molds.

Acceptance criteria for microorganism recovery was expected to be between 50-200% compared to counts on non-irradiated TSA control.

Cosmetic Aspect

Condensation and Exudation

3P culture media was evaluated for condensation/exudation inside the plate and sleeve packaging over the shelf-life of the product at both 2-8°C and room temperature (RT) storage using the qualitative and quantitative scale outlined in Table 7.

Table 7. Visible condensation and exudation scoring scale

Condensation Score	Description	Droplet Diameter	Picture
0 – Dry	No visible fog or water droplets	0	
1 – Fog and / or Tiny Droplets	Presence of fog or foggy patches with no separate drops of water	<1mm	
2 – Small Drops	Distinguishable small droplets	1 – 4mm	
3 – Full Drops	One or more drops larger than 4mm	>4mm	
4 – Water	Droplets pooling to form large areas of visible moisture	large connected droplets (Pooling of water)	

Dehydration (Resistance to Laminar Flow)

Settle plate formats have a 30 mL fill volume optimized for air sampling applications (active & passive) to reinforce resistance to dehydration effects.

To mimic passive air sampling, TSA 3P IR and TSA NEUT 3P IR were left to dry for 4.5 hrs under vertical laminar flow and then incubated 4 days at 20-25°C followed by 3 days a 30-35°C.

Dehydration was assessed over the shelf-life of the product using the scale shown in Figure 1.

Figure 1. Dehydration evaluation scale

RESULTS AND DISCUSSION - VERIFICATION

Microbiological Performance

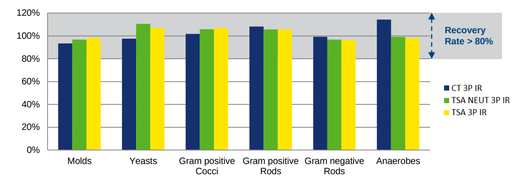

Figure 2. Average percent recovery for all strains tested per microorganism type for each 3P SMART reference

Excellent growth promotion performance for all 3P SMART references confirmed by testing a wide array of microbial strains to enhance detection of any contamination during environmental monitoring.

Neutralization Capability

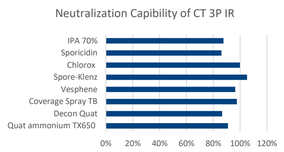

Figure 3. Average recovery rate for all strains tested against each disinfectant during CT 3P IR neutralization efficacy test

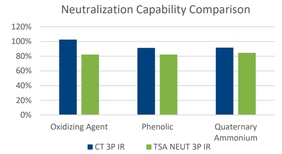

Figure 4. Average recovery rate for all strains tested against 3 disinfectant types on CT 3P IR & TSA NEUT 3P IR

CT 3P IR and TSA 3P with Neutralizers demonstrate effective neutralization for all disinfectants tested. Both products are able to neutralize various disinfectants and recover pharmacopoeia and supplemental strains within 50-200% percent recovery.

Isolator Compatibility

Specific 3P media packaging has been designed to be resistant to Vaporized Hydrogen Peroxide (VHP) cycle typically performed to decontaminate all materials being passed into the isolator.

Vaporized Hydrogen Peroxide (VHP)

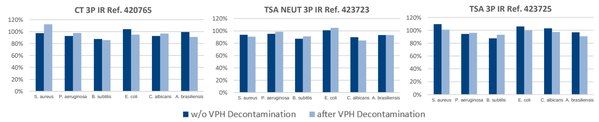

Figure 5. Percent recovery rate observed before (w/o) and after VHP decontamination cycle for all 3P SMART references

Similar growth performance of the media without and with VHP decontamination demonstrate no organisms were inhibited by VHP exposure. All 3P SMART references are validated with VHP cycle for isolator compatibility.

STABILITY – (Shelf-life Validation)

Microbiological Performance

Consistent growth recovery for all 3P SMART references through the end of shelf-life.

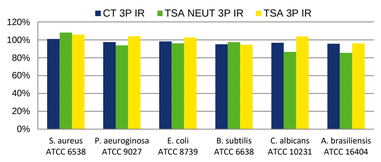

Figure 6. AVG percent recovery for all 3P SMART references through expiration

Neutralization Capability

Only two disinfectant types were tested through the end of stability for both CT 3P IR and TSA NEUT 3P IR: phenolic and quaternary ammonium compounds.

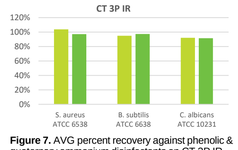

Figure 7. AVG percent recovery against phenolic & quaternary ammonium disinfectants on CT 3P IR

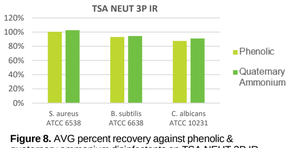

Figure 8. AVG percent recovery against phenolic & quaternary ammonium disinfectants on TSA NEUT 3P IR

High neutralization performances for CT 3P IR and TSA NEUT 3P IR to obtain good growth recovery against phenolic and quaternary ammonium disinfectants through the end of shelf-life.

Cosmetic Aspect

Condensation and Exudation

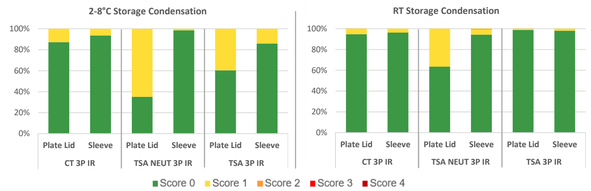

Figure 9. Condensation overview for 3P SMART references inside plate lid and innermost sleeve when stored at 2-8°C & RT

Through the end of the stability study, all 3P SMART plates demonstrate minimal condensation formation inside plate lids and innermost sleeve at either storage temperature.

Dehydration – Resistance to laminar flow exposure

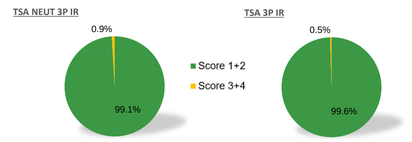

Figure 10. Cosmetic defaults overview for TSA NEUT 3P IR post dehydration process

Figure 11. Cosmetic defaults overview for TSA 3P IR post dehydration process

TSA 3P with and without neutralizers in settle plate format demonstrate robust media behavior with no major signs of dehydration during passive air sampling throughout its shelf-life duration.

CONCLUSION

Performances of 3P SMART culture media during development demonstrate excellent biological and physicochemical behavior with consistent proven product performance up to the end of shelf-life.

In addition to successfully support growth on an array of microorganisms, neutralization efficacy across different disinfectants, VHP compatible during isolator decontamination, bioMérieux guarantees:

- Completely controlled manufacturing process ensuring consistent reliability between all batches and monolot capacity.
- Maximum convenience for storage and inventory management thanks to a flexible storage between 2-25°C.
- Innovative packaging system that enables a long shelf-life.
- Security of your crucial EM samples during critical transport and incubation steps with bioMérieux's unique LOCKSURE® closure system design.
- Support data integrity and traceability solutions with GS1 data matrix barcode.

- Equivalent performance to the original 3P® with video-ink jet marking:

	Contact Plate		90 mm Settle Plate			
	3P	3P SMART	3P	3P SMART	3P	3P SMART
	CT3P Ref. 410250/418049	CT 3P IR Ref. 420765	TSA3PN Ref. 419013/419014	TSA NEUT 3P IR Ref. 423723	TSA3P Ref. 410251/418140	TSA 3P IR Ref. 423725
Appearance	✓	✓	✓	✓	✓	✓
pH						
Growth Promotion					NA	NA
Neutralization Activity						

3P SMART culture media represents the state of the art in culture media technology from both a formulation and packaging perspective allowing bioMérieux to offer the most comprehensive, robust, and reliable solution for environmental monitoring.

REFERENCES:

1. USP <1116> Microbiological Control and Monitoring of Aseptic Processing.
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5. USP <61> Microbial Examination of Nonsterile Drug Products: Microbial Enumeration Tests.