Table 17: Comparison of *Salmonella spp. Methods*

methods	Principle	Time to resolve	Confirmation	Approximate consumables cost for a batch of 50 tests (Euro)	Capital outlay	Typical Throughput	Robustness /10	Sensitivity	Competitive Flora	High Solids
CEN TC308/WG1/TG5, (2003d) Characterisation of Sludges - Part 1 Detection of Salmonella spp. - membrane filtration method for quantitative resuscitation of sub lethally stressed bacteria (6 log drop)	Diluted sludge sample filtered, and incubated at 36°c in tetrathionate broth to resuscitate for 24hrs Membrane recovered, incubated at 36°c on Rambach agar Membranes are examined after 24hrs and 48hrs (for S.Dublin) Positive colonies are quantified, salmonella indicated by red colonies resulting from fermentation of propylene glycol, some produce b-galactosidase which hydrolyses x-gal to a blue chromophore	Incubate at 48 hrs or 72 hrs (*s.dublin*) at 36°C	Spraying colonies with 1mg/ml 4-methylumbelliferyl caprylate, fluoresecent colonies confirmed as Salmonella spp. Biochemical and Serological tests.	Tetrathionate broth 30E / 500g Rambach agar 40E / 500g Petri dishes 65E per box Api 20E 125E for 25 strips **Approx. cost 30E**	Manifolds 2000E Cup and Bases 1000E Incubators 3000E Homogeniser 1750E Pipettes 150E Boiling Bath 520E Vacuum Pump 750E	Typically a sample can be processed in 48hrs and confirmed in 96hrs	7	+	+ +	+
CEN TC308/WG1/TG5, (2003e) Characterisation of Sludges - Part 2 Liquid enrichment method in selenite cysteine medium followed by Rappaport Vassiliadis for semi quantitative MPN Determination.	Sample preparation suitable for a solid matrix A selective pre enrichment step to eliminate interfering bacteria Three series of three tubes with serial dilutions for MPN format enumeration Culturing of bacteria in primary selective medium Selective Enrichment Preparation of pure cultures inoculating special solid media	Culture tubes incubated at 42°C for 20hrs. incubated on XLD and Rambach agar at 36°C for 20 hrs	Identification by biochemical and serological tests	Selenite cysteine 20E /10ml (24) Rappaport-Vassiliadis 30E / 500g XLD 50E / 500g Rambach agar 40E /500g Culture tubes 50E per box Api 20E® 125E for 25 strips **Approx. cost 35E**	Incubator 3000E Pipettes 150E Homogeniser 1750E	Typically a sample can be processed in 48hrs and confirmed in 96hrs	6	+	+ +	+ +
CEN TC308/WG1/TG5, (2003f) Charcterisation of Sludges - Part 3 Presence / Absence method by liquid enrichment in peptone novobiocin medium followed by Rappaport Vassiliadis	Four Stages of Detection Culturing of bacteria in a primary selective medium Enrichment in a selective medium which inhibits growth of other micro organisms but promotes Salmonella Preparation of pure cultures by innoculating special solid media with subcultures	Incubate in BPW with novobiocin at 36°C for 20 hrs Incubate on XLD at 36°C for 20 hrs	Identification by morphological, biochemical, and serological tests	BPW 20E / 90ml (10) Novobiocin 85E / 10ml (100) XLD 50E / 500g Api 20E 125E for 25 strips Culture tubes 50E per box **Approx. cost 65E**	Incubator 3000E Pipettes 150E Homogeniser 1750E	Typically a sample can be processed in 48hrs and confirmed in 96hrs	7	+ +	+ +	+ +
SCA (2003f) The Microbiology of Sewage Sludge (2003) - Part 4 - Methods for the detection, isolation and enumeration of Salmonella - The detection of Salmonella spp. by use of a presence/absence technique.	Isolation and detection is based on appropriate homogenization of sludge, followed by a pre-enrichment involving incubation in a non-selective medium (to recover environmentally-stressed organisms), and selective enrichment with subculture to a selective agar containing xylose with additional indicators of acidity and H2S production.	Incubate in BPW with novobiocin at 36°C for 20 hrs Incubate on XLD at 36°C for 20 hrs	Characteristic colonies are confirmed by biochemical tests and serological tests based on slide agglutination	Tetrathionate broth 30E / 500g Rambach agar 40E / 500g Petri dishes 65E per box Api 20E 125E for 25 strips **Approx. cost 20E**	Incubator 3000E Pipettes 150E Homogeniser 1750E	Typically a sample can be processed in 48hrs and confirmed in 96hrs	7	+ +	+ +	+ +
SCA (2003g), The Microbiology of Sewage Sludge (2003) - Part 4 - Methods for the detection, isolation and enumeration of Salmonella - The detection and enumeration of Salmonella spp. by a MPN technique	Isolation and enumeration is based on appropriate homogenisation of sludge, followed by multiple tube pre-enrichment involving incubation in a non-selective medium (to recover environmentally-stressed organisms), and selective enrichment with subculture to selective agar containing lactose and an indicator of acidity. The most probable number of organisms in the sample is estimated from the appropriate probability tables	Incubate in BPW with novobiocin at 36°C for 20 hrs Incubate on XLD at 36°C for 20 hrs	Characteristic colonies are confirmed by biochemical tests and serological tests based on slide agglutination	Selenite cysteine 20E /10ml (24) Rappaport-Vassiliadis 30E / 500g XLD 50E / 500g Rambach agar 40E / 500g Culture tubes 50E per box Api 20E® 125E for 25 strips **Approx. cost 60E**	Incubator 3000E Pipettes 150E Homogeniser 1750E	Typically a sample can be processed in 48hrs and confirmed in 96hrs	6	+ +	++	+ +
SCA (2003h), The Microbiology of Sewage Sludge (2003) Part 4 - Methods for the detection, isolation and enumeration of Salmonella- The enumeration of Salmonella spp. by a membrane filtration technique with resuscitation and culture on a chromogenic detection medium	Diluted sludge sample filtered, and incubated at 36°c in tetrathinate broth to resuscitate for 24hrs Membrane recovered, incubated at 36°c on Rambach agar Membranes are examined after 24hrs and 48hrs (for *S.Dublin*) Positive colonies are quantified, salmonella indicated by red colonies resulting from fermentation of propylene glycol, some produce b-galactosidase which hydrolyses x-gal to a blue chromophore	Incubate at 48 hrs or 72 hrs (*s.dublin*) at 36°C	Salmonella colonies exhibit fluoresence when exposed to UV light at 366nm.	BPW 20E / 90ml (10) Novobiocin 85E / 10ml (100) XLD 50E / 500g Api 20E 125E for 25 strips Culture tubes 50E per box **Approx cost 75E**	Manifolds 2000E Cup and Bases 1000E Incubators 3000E Homogeniser 1750E Pipettes 150E Boiling Bath 520E Vacuum Pump 750E	Typically a sample can be processed in 48hrs and confirmed in 72hrs	7	+	+ +	+
Matrix MicroScience (2003) Detection of Salmonella using Immunomagnetic Seperation using Pathatrix®	Enrichment. A 25 -27g portion of the sample is weighed in a stomacher bag and Buffered Peptone Water added to make a 1 in 10 dilution. This processed using the stomacher for 30secs. To this is added antiserum coated beads, and the bag is incubated on the Pathatrix Equipment at 37°c for 3 hours. The beads are recovered and rinsed, and the concentrated bead suspension is plated on to the selective medium. This is incubated at 37°c for 24 hours.	Incubated on the Pathatrix system for 3hrs at 37°C Incubated on the selective medium for 24hrs at 37°C	Confirmed by flurotrix (fluorescent microscopy) and serology	BPW 20E / 90ml (10) Antiserum coated beads **Approx cost 400E**	Pathatrix® System Gravimetric diluter Incubator 3000E	Identification in 16 hrs	8	+ + +	+ + +	+ + +
Merck Singlepath	See section 2.5.5 in the report			**Approx device cost for 50 samples 350E**		2 d to confirmed result	8	+ + +	+ + +	+ +

N.B It should be noted that all methods will also require the use of an autoclave (steam steriliser) to make relevant media for use in the methods (approx. price 40,000E)
All prices quoted are in Euros
Numbers in brackets are related to the pack size of the product

Table 18 - Comparison of *Escerichia Coli Methods*

Method	Principle	Time to resolve	Confirmation	Approximate consumables cost for a batch of 50 tests (Euro)	Capital outlay	Typical Throughput	Robustness /10	Sensitivity	Competitive Flora	High Solids
CEN TC308/WG1/TG5(a) Characterisation of Sludges - Detection of *Escherichia Coli* Part 1: Membrane Filtration for Quantification	10g Sludge into 90ml MRD (PS) Serial Dilution 1ml PS - 9ml MRD TO 10^{-7} Membrane Filtration through 0.45mm membrane	Incubate on MLGA at 30°C 4hrs / 44°C for 14 hrs	Confirm on API 20E if requested, not usually required	Membranes 70E per box MLGA (Oxoid) 120E per tub /500g Petri dishes 65E per box (1620) **Approx. cost 40E**	Manifolds 2000E Cup and Bases 1000E Incubators 3000E Homogeniser 1750E Pipettes 150E Boiling Bath 520E Vacuum Pump 750E	Dilutions to make up, filtering relatively quick 20 samples per hour. Confirmed result within 18 hrs of filtration	5	+ + +	+ +	+
CEN TC308/WG1/TG5 (b) Characterisation of Sludges - Detection and Enumeration of Sewage Sludges - Miniaturised method (MPN) in Liquid Medium	10g Dry Matter Sludge into a tryptone diluent final volume of 100ml (PS) Mix 2ml of PS with 18ml of Special Diluent Prepare Serial Dilutions in Special Diluent e.g. 1/10 - 1/200 000 Inoculate the microplate	Incubate at 44°C for 36hrs minimum and 72 hrs maximum	The presence of E.coli is indicated by a blue fluorescence resulting from the hydrolysis of MUG	Microplate 55E per plate Tryptone salt diluent 20E /500g Dehydrated culture medium Culture tubes 50E per box MUG Lauryl sulphate broth 75E / 500g **Approx. cost 260E**	Incubators 3000E Homogeniser 1750E UV-lamp (366 nm) 300E Laboratory shaker 1500E Pipettes 150E	Samples take approximately 24 hrs to be processed Confirmed results within 48 hrs	7	+ +	+	+ + +
CEN TC308/WG1/TG5(c) Characterisation of Sludges - Detection and Enumeration of Escherichia Coli from Sewage Sludge Part 3: Macromethod (MPN) in Liquid Medium	Place 20g wet weight of sample into 180ml 0.9% sterile NaCl solution Prepare serial tenfold dilution 1ml of PS + 9ml 0.9% NaCl upto 10^{-7} From each dilution step transfer 1ml per tube into 3 tubes containing MUG Fluorocult LSB each	Incubate for 40hrs at 44°c	To read add 0.5ml NaOH to each tube and observe for fluoresence with a 366nm UV light	Culture tubes 50E per box NaCl 10E/ 500g NaOH 30E/ 250g MUG Lauryl sulphate broth 75E/ 500g **Approx. cost 20E**	Incubators 3000E UV-lamp(366 nm) 300E Pipettes 150E Homogeniser 1750E	Samples take approximately 24 hrs to be processed Confirmed results within 48 hrs	8	+ + +	+ +	+ + +
ISO 16649-2: Detection and enumeration of Escherichia coli - membrane filtration method using Chromocult® agar	10g Sludge into 90ml of MRD (Primary Suspension) Serial Dilutions of 1ml PS - 9ml MRD to 10^{-7} Membrane filtration through 0.45mm membrane Escherichia Coli colonies are blue -green (X-b-D glucuronide reaction)	Incubate at 30°C for 4hrs and then 44°C for 18-20 hrs	Confirmation of blue/ green colonies not required due to the specificity of the media	Chromocult® agar 120E per tub Membranes 70E per box Petri dishes 65E per box (1620) **Approx. cost 40E**	Incubators 3000E Manifolds 2000E Cups and Bases 1000E Homogeniser 1750E Boiling bath 520E Vacuum Pump 750E	Dilutions to make up, filtering relatively quick 20 samples per hour. Confirmed result within 20 hrs	5	+ +	+ +	+
SCA (2002) The Microbiology of Drinking Water - Part 4: Detection and enumeration of Escherichia coli -membrane filtration method using MLSA agar	10g Sludge into 90ml MRD (Primary Suspension) Serial Dilutions of 1ml PS - 9ml MRD to 10-7 Membrane filtration through 0.45mm membrane onto MLSA agar	Incubation at 30°C for 4hrs and then 44°C for 14 hrs	Confirmation through the production of acid from lactose, negative oixdase reaction and indole formation	MLSA agar 120E per tub /500g Membranes 70E box Petri dishes 65E per box (1620) **Approx. cost 40E**	Incubators 3000E Manifolds 2000E Cups and Bases 1000E Homogeniser 1750E Boiling bath 520E Vacuum Pump 750E	Dilutions to make up, filtering relatively quick 20 samples per hour. Confirmation within 72 hrs	4	+	+	+
SCA (2002) The Microbiology of Drinking Water - Part 4: Detection and enumeration of Escherichia coli - membrane filtration method using MLSB agar	10g Sludge into 90ml MRD (Primary Suspension) Serial Dilutions of 1ml PS - 9ml MRD to 10-7 Membrane filtration through 0.45mm membrane onto MLSB agar	Incubation at 30°C for 4hrs and then 44°C for 14 hrs	Confirmation through the production of acid from lactose, negative oixdase reaction and indole formation	MLSB 40E per tub /500g Membranes 70E per box Petri dishes 65E per box **Approx. cost 30E**	Incubators 3000E Manifolds 2000E Cups and Bases 1000E Homogeniser 1750E Boiling bath 520E Vacuum Pump 750E	Dilutions to make up, filtering relatively quick 20 samples per hour. Confirmation within 72 hrs	4	+	+	+
Andrews and Presnell (1990): The A-1 method Greater Vancouver Regional Council (GVRD): Multiple Tube Fermentation (MTF) technique to detect and enumerate Escherichia coli in biosolids	Similar to MTF analysis (MPN) technique Sample homogenised and serial dilutions taken to 10-7 Uses A-1 media, no requirement for enrichment step using LTB	Samples conditioned at 35°C for 3hrs and placed in a faecal water bath at 44.5°C for 21 hrs	A-1 media does not require confirmation due to its specificity	A-1 Media 130E per box Culture tubes 50E per box **Approx. cost 30E**	Incubators 3000E Water Bath 750E Homogeniser 1750E Pipettes 150E	Samples take approximately 24 hrs to be processed Confirmed results within 24hrs	6	+ +	+ +	+ +
Colilert® IDEXX Laboratories Ltd. (2003) IDEXX Colilert® Method - Enumeration of coliform and Escherichia coli bacteria in waste water solids using defined substrate technology®	Sample Homogenised and 50ml added to 450ml of sterile, buffered dilution water Serial dilutions prepared - 20 replicates, dilution A (0.001), B (0.00001), C (0.0000001) Take 100ml of A,B,C mix with colilert media, add to quantitray package, seal and incubate	35°C for 24hrs	No confirmation required, colour change indicates presence of E.coli	Quantitray Packs 100ml containers Colilert® media **(Awaiting prices from IDEXX)**	Incubators 3000E Quantitray sealer UV Lamp 300E Homogeniser 1750E Pipettes 150E	Less than one minute per sample test Confirmed result within 24 hrs	8	+ + +	+ +	+ + +
U.S. EPA - MTF technique for the detection and enumeration of Escherichia coli in waster activated solids EPA-600/8-78-017.	Sample Homogenised and 50ml added to 450ml of sterile, buffered dilution water Serial dilutions prepared - 20 replicates, dilution A (0.001), B (0.00001), C (0.0000001) Presumptive Phase - 4 groups of 5 tubes incubated at 35°c Various dilution added to tubes containing LTB Confirmed Phase - using Brilliant Green LTB, Completed phase using EC-MUG media	Incubated at 35°for 48 hrs and then completed phase incubated at 44.5°C for 24hrs	Confirmation using brilliant green LTB and EC MUG media	Culture tubes 50E per box Lauryl Tryptone Broth 50E / 500g Brilliant Green LTB 60E / 500g EC-MUG Media 75E / 500g **Approx. cost 40E**	Incubators 3000E Homogeniser 1750E UV Lamp 300E	72 hrs before a confirmed result	7	+	+ +	+ +
Microbiology of Sewage Sludge - Part 3 Method A (2003) isolation and enumeration of Escherichia coli using a chromogenic membrane filtration technique	Sample homogenised, serially diluted with MRD and filtered through membrane filter and placed onto an agar plate of MLGA media. Colonies that are β-glucuronidase positve and ferment lactose are considered as E.coli	Incubation at 30°C for 4hrs and then 44°C for 14 hrs	Confirmation not required	MLGA Media 70E per tube Petri dishes 65E per box Membranes 70E per box **Approx. cost 40E**	Incubators 3000E Manifolds 2000E Cups and Bases 1000E Homogeniser 1750E Boiling bath 520E Vacuum Pump 750E Pipettes 150E	Dilutions to make up, filtering relatively quick 20 samples per hour. Confirmed result within 18hrs of filtration	5	+ + +	+ +	+
Microbiology of Sewage Sludge - Part 3 Method B (2003) isolation and enumeration of Escherichia coli using a multiple tube fermentation MPN technique	10g sample homogenised in MRD and added to a series of tubes containing liquid enrichment broth. Positive tubes are sub-cultured onto confirmation media.	Selective enrichment at 36°C for 24 hrs and then at 44°C for 24hrs	Growth at 44°C in the presence of brilliant green with the production of gas from lactose and the formation of indole	Brilliant Green Bile Broth 55E / 500g Tryptone Water 30E / 500g Sodium Lauryl Sulphate Bromocresol purple Culture tubes 50E per box **Approx. cost 30E**	Incubators 3000E Homogeniser 1750E		7	+ +	+	+ + +
Microbiology of Sewage Sludge - Part 3 Method C (2003) isolation and enumeration of Escherichia coli using defined substrate technologyÒ for MPN determination (Similar to Coliert method)	Samples are homogenised and serially diluted with MRD. The samples are incubated in a defined liquid medium containing specific substrates for the detection of the enzymes β-galactosidase and β-glucuronidase. The samples is then added to a Quantitray® pouch and incubated.	Incubate at 37°C for 18 hours	No confirmation required	Quantitray Packs 100ml containers Colilert® media **(Awaiting prices from IDEXX)**	Incubators 3000E Quantitray Sealer Homogeniser 1750E	Less than one minute per sample test	8	+ + +	+ +	+ + +

N.B It should be noted that all methods will also require the use of an autoclave (steam sterilser) to make relevant media for use in the methods (approx. price 40,000E)
All prices quoted are in Euros
Numbers in brackets are related to the pack size of the product