not significantly contaminated. Conversely, if significant contamination is found in any of the selected sample points, it indicates a potential for problems, and the results should be acted on at the earliest convenient opportunity.

The monitoring programme should be consistent in the selection of either water or bottom fuel phase samples as tests results of the two phases are not always directly comparable. Generally, levels of contamination in any water phase in a tank will remain more consistent than those in the fuel phase just above it. Water phase will also contain far higher levels of microbial contamination than fuel, and therefore routine testing of bottom water phase will enable earlier detection of growth. However, detection of contamination in water does not necessarily mean contamination will adversely affect the fuel. Also, water phase samples are often not readily recoverable for routine testing, particularly in well-maintained facilities where water is drained off regularly. Therefore, in many cases, it is more appropriate to routinely monitor dead bottom fuel phase samples, even if water phase samples can occasionally be recovered. It should be appreciated that microbiological contamination levels in fuel phase samples will typically decrease with increasing height above the water phase; for example, a fuel sample taken from the dead bottom immediately above any free water will contain far higher numbers of microbes than a sample taken, say, 30 cm above the tank bottom. Again, the importance of consistency in selecting sampling locations for routine monitoring is stressed; any microbial limit values set (see 5.2.5) should take sampling location into consideration.

Occasional tests of bulk fuel samples (e.g. from tank delivery points, suction level or fuel upper, middle and lower layers) may be conducted if there is reason to suspect presence of contamination, or if there is a specific need to assess the impact of possible contamination of the fuel as a whole. Different microbial standards should be applied to bulk fuel layer samples than are applied to bottom or low point samples, and it should be appreciated that the contamination in fuel phase will be more prone to variation, depending on the degree of disturbance in the tank or any settling time applied prior to sampling.

Details of procedures for sampling and testing are described in 5.6.

5.2.5 Actions in response to test data

A well-designed routine monitoring programme will include predetermined actions to be taken in response to the test data. The results of tests should be amenable to categorisation of the contamination detected, for example:

- Acceptable: contamination is not above levels which are normally encountered; no further action until the next routine test.
- Moderate/warning: there is indication that some microbial proliferation is occurring. Review control procedures.
- Heavy/action: microbiological proliferation is occurring, and heavy contamination is present. Investigation and remedial actions are required.

Guidance on defining the contamination categories for microbiological tests of fuel and associated water is given in 5.7, where the factors which should be considered when interpreting test data are considered in more detail. It is important to appreciate that even clean fuel, and water associated with fuel, contains a few microbes. But when active microbial proliferation is occurring, far higher numbers of microbes can be recovered and these will be generally indicative of the extent of microbial proliferation.

Trends of contamination are as important as absolute numbers. When setting up routine monitoring programmes, it is useful to establish broad baseline contamination levels during the initial risk assessment, i.e. those which can be tolerated without causing fuel quality issues or operational problems. Different facilities and systems will tolerate different levels of contamination and there can be differences in contamination between sampling locations. It is also not unusual to detect variation in the level of microbial contamination found in a single sampling location at different times. Two- or three-fold differences in microbial counts are generally not significant, whereas differences of 10-fold or more are significant.

Figure 18 shows data for monthly bottom fuel samples from a diesel storage tank. Whilst there is month-to-month variation in results, an overall trend of increasing contamination is apparent on the last three sampling occasions; this would instigate investigative and, if appropriate, remedial action.

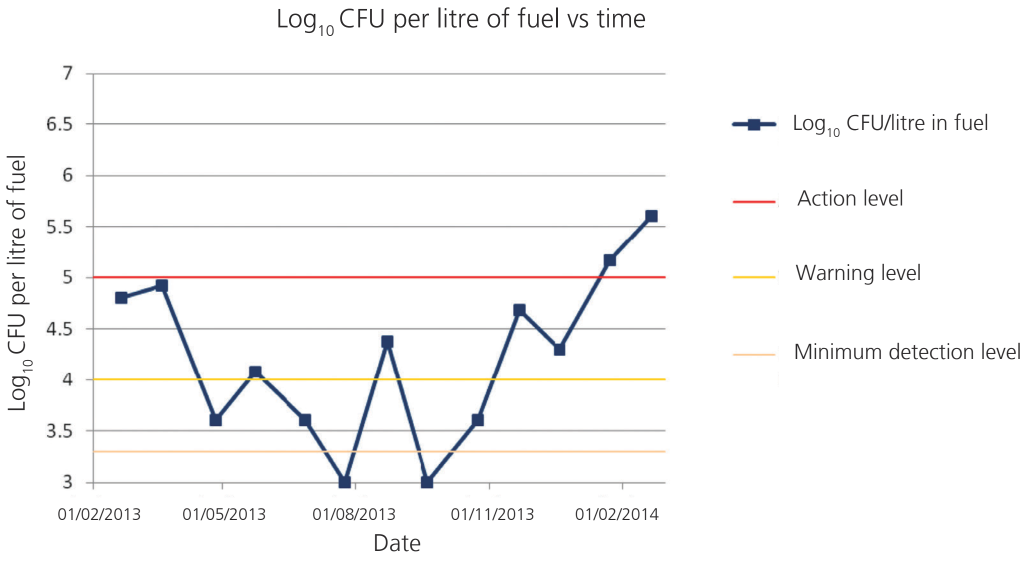

Figure 18: Typical test data for routine monthly monitoring (IP 613) of bottom fuel samples from a diesel storage tank

Detection of a 'heavy/action' contamination level in a bottom sample, and even in a bulk fuel sample, does not necessarily mean that fuel from the facility is unfit for use but it is potentially serious and should not be ignored. In some cases, transient contamination associated with a particular batch of fuel may be encountered, but usually a heavy contamination is unlikely to resolve itself. In most cases, the first appropriate action will be to resample and retest to confirm the result was not due to a transient contamination or test error. Further details on remedial measures are provided in section 6.

Figure 19 shows typical responses to microbiological test results and is applicable to most fuel types and facilities. 5.2.6 highlights additional considerations for routine monitoring for specific fuel types and facilities.

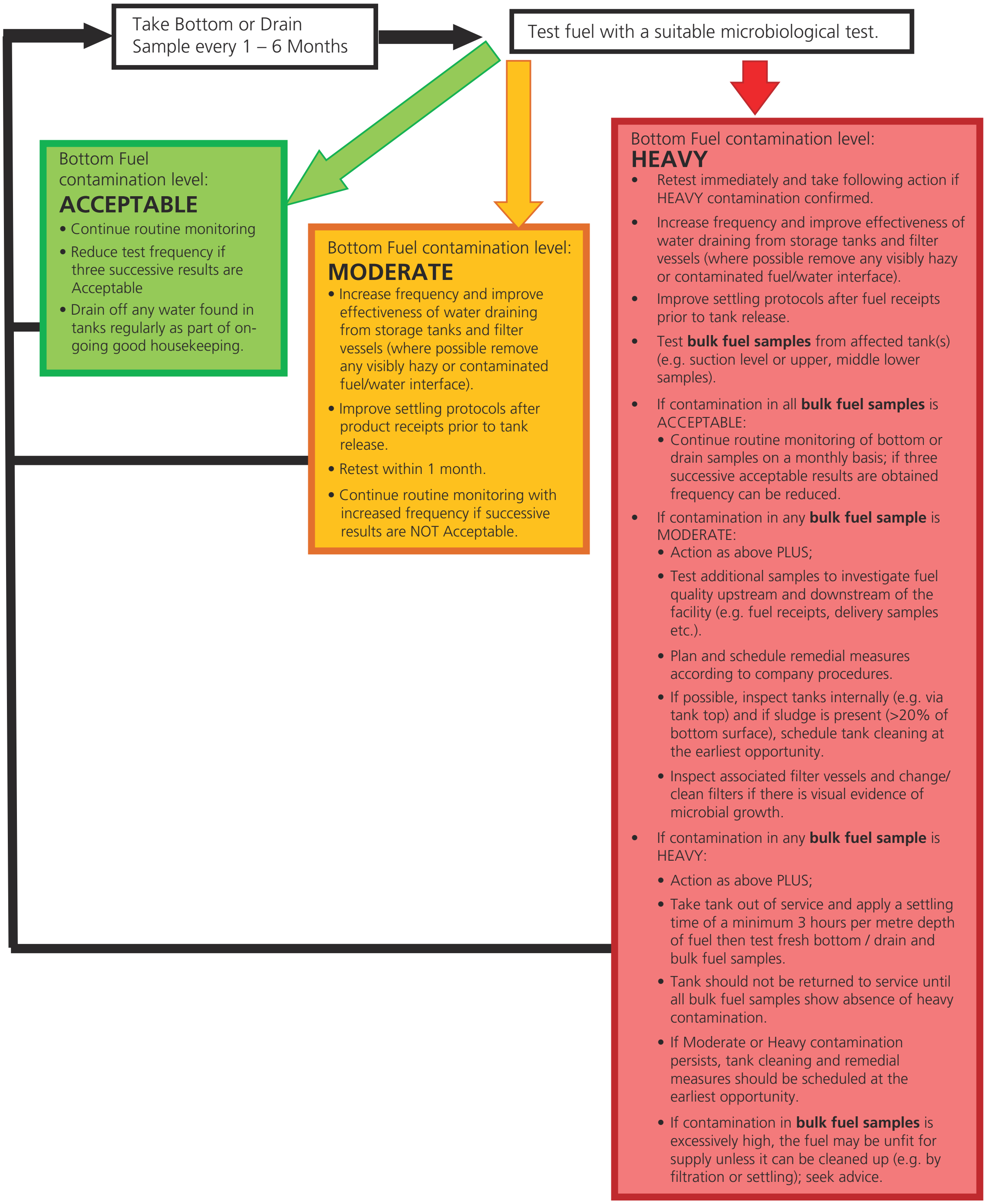

Figure 19: Flow diagram of typical microbial monitoring programme

5.2.6 Routine microbial monitoring for specific fuel types

5.2.6.1 Diesel fuel

Information on the problems caused by microbial contamination in diesel is provided in 3.4.1.

Monitoring supply and distribution network (refinery, terminal and diesel fuel storage tanks, pipelines and transportation tanks):

– It is recommended that where routine housekeeping practices cannot keep microbial growth within acceptable limits, routine microbial monitoring of tank bottom, lower level or drain samples (see 5.2.4) takes place on a scheduled basis. Typically, this will be conducted every one to six months, depending on the tank's history (water ingress, fouling, corrosion, etc.), the effectiveness of water draining and product settling practices and the height of the tank suction level (i.e. the risk of delivering fuel contaminated with microbial growth from the tank bottom). Tanks storing diesel with FAME at concentrations between 2 – 20 % will typically be monitored monthly. Tanks storing FAME (B100) or FAME blends of greater than B20 will generally only require routine testing if free water is known to accumulate in them.
– A suction level sample or bulk fuel layer samples may additionally be tested when microbial test of a bottom or drain sample (see 5.2.5) indicates the presence of heavy contamination. Ideally, these samples should be taken immediately after any standard settling protocols have been applied, to best reflect the microbial content in fuel delivered from the tank.

Monitoring diesel retail sites and end-user diesel storage tanks (e.g. utilities, rail and vehicle fleet operators):

– Some operators of retail site diesel tanks or end-user depot tanks employ routine microbial monitoring to highlight the need for tank cleaning, fuel polishing or biocide treatment, particularly where there is a history of contamination of fuel tanks of vehicles using the facility or where it is very difficult to control accumulation of water (e.g. underground storage tanks). The sampling and testing will typically be conducted every three to 12 months depending on the perceived risk. Usually, a tank low point sample is tested but a sample from the pump delivery nozzle can also be tested to provide assurances about the levels of contamination in fuel delivered to vehicle tanks.

5.2.6.2 Aviation fuel

Information on the problems microbial contamination causes in aviation turbine fuels is provided in 3.4.2.

Monitoring supply and distribution network (refinery, terminal and airport fuel storage tanks, pipelines and transportation tanks):

– EI/JIG 1530, JIG 1, JIG 2 and JIG 4 recommend the use of the IATA recommended test kits to establish baseline levels of contamination in storage tank bottoms and, where appropriate, ongoing routine monitoring at a frequency dependent on the perceived risk and/or historical incidence of microbial growth. JIG *Microbial monitoring strategies* provides details on the process of risk assessment and states that facilities should be considered:
  – 'High risk' if at any time in the previous two years, 'action level' microbial contamination has been detected at any sampling location on more than one occasion, or if significant microbial growth has been observed during inspection

of tanks or filters. Monthly microbiological testing of fuel from the storage tank drains recommended.

- 'Moderate risk' if there has been a single incident of 'action level' microbial contamination detected at any sampling location in the previous two years and/or if the facility operates under conditions which may be conducive to microbial growth (e.g. facilities in hot, humid environments, facilities where water or dirt is known to ingress or accumulate in tanks, facilities which are ship-fed and facilities undergoing engineering works such as hydrant installation or repairs). Quarterly microbiological testing of fuel from the storage tank drains recommended.
- 'Low risk' if the facility does not operate under conditions specifically conducive to microbial growth and no samples have shown 'action level' contamination and there have been no other indications of microbial growth in the previous two years. Some limited sampling and testing of these facilities (e.g. annually) might be advisable.

- The fuel samples drawn from tank drains should be taken after standard line flushing procedures have been applied to remove water or hazy product. For moderate and high-risk facilities additional investigative testing can be warranted of low point or drain samples from receipt and outlet filter vessels, hydrants, pipelines, refuellers and hydrant dispensers; this testing should be conducted on an ongoing basis for two years following return of contamination to low level.
- In the USA, API RP 1595 takes a slightly different approach to the requirement for routine microbial testing and stipulates a minimum six-monthly check of drain or bottom samples from all storage tanks.
  - Although the routine testing described in this section is recommended, JIG Standards only mandate the use of semi-annual microbial monitoring for vehicles routinely used for the defuelling of aviation fuel. Microbial monitoring may also be used as an alternative to quarterly visual inspections to assess the microbiological cleanliness of product recovery tanks and to evaluate possible extension to the main storage tank cleaning frequency.

Monitoring aircraft fuel tanks:

- Although fuel tanks can be inspected internally during maintenance checks, there is no practical method of viewing tank surfaces whilst an aircraft is in service and if growth is occurring on them a test on a drain sample is the only test which can be made. IATA's Guidance Material recommends at least annual microbial testing of a drain sample from each fuel tank using specified recommended tests (see 5.5.2). The recommendations are adopted in the AMM of most major aircraft manufacturers. More frequent testing is recommended for aircraft operating under conditions more conducive to microbial growth, for hot humid climates, low altitude short haul operations.

5.2.6.3 Marine distillate fuels and fuels for offshore power generation

Information on the problems microbial contamination causes in marine fuels is provided in 3.4.3.

Monitoring supply and distribution network (refinery, terminal and marine fuel storage tanks and pipelines and transportation tanks):

- It is recommended that where routine housekeeping practices cannot keep microbial growth within acceptable limits, routine microbial monitoring of tank bottom, lower level or drain samples (see 5.2.4) takes place on a scheduled basis. The considerations are as discussed for diesel fuel in 5.2.6.1.

Ships' bunkers and fuel tanks on-board:

– It is common practice for ship and platform operators to subscribe to a routine bunker analysis service and there are many global providers. Although not stipulated in marine fuel specifications, some ship operators choose to include checks for microbial contamination in the suite of tests conducted on marine distillate fuels. Additional routine microbiological testing of drain samples from on-board fuel tanks such as settling tanks, day tanks and auxiliary tanks for generators, lifeboats, cranes and other equipment can establish whether microbial growth is occurring after bunkering fuel. Many platform operators and some ship operators have an onboard monitoring programme utilising either contract laboratories or field test kits. Where there is a chance that fuel containing FAME is bunkered, there can be a strong case for routine onboard microbial testing as a precautionary measure. Typically, this will be conducted on a monthly to quarterly basis.

5.2.6.4 Gas oil, heating oil and other middle distillate fuels

Information on the problems microbial contamination causes in gas oil, heating oil and other middle distillate fuels is provided in 3.4.4.

Monitoring supply and distribution network (refinery, terminal and fuel storage tanks, pipelines and transportation tanks:

– It is recommended that where routine housekeeping practices cannot keep microbial growth within acceptable limits, routine microbial monitoring of tank bottom, lower level or drain samples (see 5.2.4) takes place on a scheduled basis. The considerations are as discussed for diesel fuel in 5.2.6.1.

End-user storage tanks:

– These include small storage tanks used in power generation facilities, data centres, industrial and agricultural facilities, hospitals and other utilities and also domestic heating installations. Unless there has been a history of problems, routine sampling and testing is rare, although routine testing of a tank drain sample could be considered as a precautionary measure where the application is critical (e.g. data centres and hospitals), where the fuel stored contains FAME and/or if there is long-term storage in excess of three months.

5.2.6.5 Motor and aviation gasoline

Information on the problems microbial contamination causes in gasoline is provided in 3.4.5 and 3.4.6.

Routine microbiological monitoring of gasoline tanks is rarely warranted, given the relative scarcity of microbial problems in these fuel types. In the USA, where problems associated with microbial contamination of unleaded motor gasoline are more widely reported, some monitoring of water bottom samples from storage tanks at terminals and retail sites may be conducted. Testing of the hydrocarbon phase of gasoline tanks rarely, if ever, provides useful test data.

5.3 MICROBIOLOGICAL INVESTIGATION

A microbiological investigation can attempt to evaluate any of the situations identified in Table 2, or it could be undertaken to determine if there is a potential for initiating a future problem. Any sampling and evaluation of the sample is a snapshot in time and place, and an interpretation of results should consider the potential for change in the system and the sample.

Investigation of suspected microbial contamination can be initiated by a number of scenarios, including:

– visual indicators when draining tanks, for example presence of dirty water or fuel;
– visual indicators during inspections and maintenance checks, for example spotting or staining on tank surfaces/filters and the presence of slime;
– visual indication of contamination in fuel on delivery (e.g. discharge of cargoes);
– operational problems such as filter clogging, coalescer failures and fuel gauge malfunction, and
– as a response to results of routine monitoring exceeding warning or action levels.

Table 2: Examples of situations which might be investigated

Sector	Type of problem caused by microbes
Refinery	Fouling in blend and finished product tanks and/or corrosion. High sulfide concentrations in product and/or tank drain water. Viable microbes at the refinery 'inoculating' vehicles and facilities in the distribution chain
Distribution	Fouling and/or corrosion in tanks, pipelines and vehicles. Clean fuel received at site, but contaminated fuel delivered from site. Filter plugging; FWS malfunction; customer complaints
Storage	Viable microbes could proliferate causing changes in product properties which could in turn result in the product failing to meet specification requirements. SRB could cause corrosion in storage tanks and/or generate hazardous H_2S gas; complaints from purchasers
End-user	Microbes in supplied fuel could affect the fuel's fitness for use, with or without further growth in end-user tanks. Problems related to fouling, filterability, surfactancy, equipment malfunction and corrosion could occur and can be ascribed to both viable and non-viable microbes. Sulfide in fuel causes corrosion and failure of copper and/or silver strip tests. Supplied product no longer meets its original specification requirement. Ongoing microbial contamination in fuel supplied prevents end-user from controlling further growth in their facility – the more microbes imported, the more difficult this is. Growth at end-user facility causes increased operational or corrosion problems

Microbial contamination is unlikely to disappear by itself and is more likely to get worse over time. If there are operational indicators, this suggests that microbial contamination is already out of control and an investigation needs to be conducted. It is recommended that professional advice and support are sought prior to and during a microbiological investigation. However, this section outlines some of the factors which need to be considered when conducting an investigation.

The investigation might consider the following:

– Are microbes present?
– Are the microbes present capable of causing the problem?
– Are microbes in sufficient numbers to cause problems?
– Where are they coming from?
– Is there a potential for a problem?
– Is a remediation strategy required?
– If yes, was the remediation strategy applied successful?
– Can avoidance measures, i.e. good housekeeping, be improved to prevent problem recurrence?

It must be noted that the detection of viable microbes does not necessarily prove that they caused the problem. The investigation should assess whether there are abnormal levels of contamination, indicative of microbial proliferation. The likely impact on product fitness can be considered in combination with the evidence of other analyses, such as microscope examination. The 'toolbox' of non-microbial and microbial analysis methods that are useful in a microbiological investigation are discussed in 5.5. The precise objectives of a microbiological investigation are ideally considered before drawing samples. If possible, the analyses to be conducted should be determined in advance, as the sampling requirements for different tests may vary. Sampling procedures and techniques are discussed in 5.6.

When conducting the investigation, microbiological analysis of a tank bottom or low point sample, and particularly any free water, interface or sludge phase, is most appropriate for determining if any significant microbial growth has occurred within a tank or system. These will be 'worst case' samples and will not represent contamination in the bulk of the fuel. However, their analysis can indicate the potential of microbial growth to contaminate the fuel passing through the tank, with consequent adverse implications for both fuel quality and dissemination of contamination down the fuel distribution chain. If samples of biomass or sludge can be obtained, for example from filters and filter casings or tanks' surfaces, it is highly recommended that these are tested as part of the investigation.

A microbiological analysis of fuel layer samples or a tank suction level sample will help to determine whether contamination in the tank bottom has any bearing on fuel quality. It is important to draw such samples after consideration of any effects of product movements and settling times. The extent of contamination in bulk fuel is likely to vary considerably both with time and with distance from the site of active microbial growth (e.g. height above the tank bottom). Therefore, it should be noted that any bulk fuel samples taken after an incident may not reflect the extent of contamination present at the time the incident occurred. For example, a fuel tank which is not given adequate settling time after a receipt could deliver heavily contaminated fuel which results in operational problems for the user but, if sampled several days after the event, the bulk fuel may be found to contain relatively low levels of contamination due to the subsequent settling of microbial contaminants.

For ships' cargo tanks, it will usually be necessary to sample and test multiple layers of each compartment as contamination in any single compartment could be the cause of contamination in the whole fuel parcel. Often investigation of fuel cargo spoilage is reliant on retrospective testing of samples drawn during and after loading, and before discharge. In the event of a bunker fuel quality dispute or operational problem on a ship, retrospective microbiological testing is sometimes conducted on retained samples drawn using proportional samplers or drip samples from a delivery line taken during bunkering. Unfortunately, these samples are likely to have been taken some considerable time before the investigation and the delay in testing can compromise the validity of the test results (see 5.5.2.2 and 5.6.6).

When investigating distribution pipelines and equipment, numerous locations can be selected for investigative sampling and testing, for example:

- pipeline and hydrant low points, where accessible;
- pigging debris;
- before and after a filter to check its ability to remove microbes;
- before and after an FWS to check the ability of the FWS to remove microbes and to confirm that the unit has not become colonised by microbial growth (increase in numbers detected);
- the water drain from a filter vessel (note: many microbes dispersed in wet fuel will actually be in the water droplets), and
- the surface swab of an FWS if the unit has been opened and 'leopard spotting' is observed or accumulated deposits on other filter types.

Where operational problems suspected to be due to microbial contamination are experienced by fuel end-users in trucks, buses, motor boats, generators, rail locomotives, tractors and other industrial vehicles, appropriate samples for investigations are from the fuel tank drain and the water settling/separation device (sediment bowl) plus the filter. Fuel end-users who are investigating microbial quality problems should always try to establish the microbial quality of fuel supplied to them. This will be the baseline for comparison with results of additional representative samples taken from appropriate points on-board their vessel, aircraft, vehicle or train. It will then be possible to determine whether operational problems have been encountered due to a quality problem with the fuel supply or due to growth on-board (or both). It should be remembered, however, that a negative result for a test of a single spot sample of the batch supplied will not necessarily provide assurances about its quality. Samples provided by the fuel supplier may not always be thoroughly representative of the fuel actually delivered.

If there are indications of high sulfide in the fuel or H_2S emanating from tank drain water, or if there is a history of tank bottom pitting corrosion, a bottom water or sludge sample should be taken and tested for SRB. If the tank is accessed for inspection, swabs of deposits in corrosion pits can also be tested for SRB to establish if they have played a role in corrosion. Simple lead acetate indicator paper tests for presence of sulfide can also be used to determine if microbially generated sulfide is present in corrosion deposits. Ideally, investigative sampling and testing for SRB should be conducted before tank cleaning removes any vital evidence which may be present in the sludge and corrosion deposits. However, it must be ensured that it is safe to enter tanks to take appropriate samples (see EI *Model Code of Safe Practice Part 16: Guidance on tank cleaning*).

Professional experts can assist in determining the source of the microbiological contamination. To do this it may be necessary for samples from various key locations in the supply chain to be tested by specialist laboratories to establish the identity of key microbial contaminants, using biochemical tests, conventional selective microbiological media or molecular microbiological methods (MMM); the latter are particularly useful for this purpose. However, it must be remembered that most fuels contain microbes in some small quantity. If, for example, fuel received from a refinery tank was found to be the likely source of microbes into a tank/system where they subsequently proliferated and caused problems, it must be remembered that the receiving tank/system offered the conditions where these microbes could grow and proliferate.

It should be noted that pigging debris, deposits from filters, and sludge from tank bottoms can be expected to contain considerable numbers of microbes even when drawn from systems which do not have an operationally significant level of microbial growth. Expert assistance may be needed in interpretation of test results of these samples and qualitative examination (e.g. microscopy to determine the nature of material present) can be more important than quantitative microbiological test data.

It is important to consult field operators when investigating a potential microbial issue. They will usually have specific experience of the facility or system, which will assist in selection of appropriate sampling locations. For example, they may be able to advise whether particular tanks are prone to slime accumulation, or comment on operating conditions that promote water accumulation.

To guarantee that those conducting the analyses can provide the best possible interpretation on the results obtained, as much information as possible about the sampling needs to be conveyed to the testing laboratory.

In the event of disputes where microbial contamination is alleged to have influenced the fuel's fitness-for-purpose, a complete range of representative samples of fuel phase, plus water phase, will ideally be tested. However, as disputes can be protracted, suitable fresh samples may no longer be obtainable when the analysis is conducted (sometimes several years after the disputed incident); in such cases, exceptional caution must be exercised when interpreting results of tests for viable organisms.

5.4 TESTING AS PART OF A HEALTH AND SAFETY RISK ASSESSMENT

Prior to tank cleaning, it may be appropriate to sample sludge from the tank bottom to establish whether pathogenic microbes (e.g. *Pseudomonas* species) are present, and whether these could pose a risk to personnel cleaning the tank. Safety assessments may also involve sampling to establish the potential for sulfide generation by SRB; appropriate sampling points will be those areas most likely to be conducive to SRB proliferation, namely, stagnant areas where water accumulates. It should be appreciated when devising the sampling plan that the point of exposure to H_2S may be downstream of the site of SRB proliferation, for example adjacent to separators receiving tank drain water.

5.5 TOOLBOX OF TEST METHODS

5.5.1 Non-microbial methods useful in investigation or assessment of microbial contamination

A variety of laboratory and field methods is available to specifically detect microbial contamination in fuel; these methods are reviewed in 5.5.2. However, several other methods, whose purpose is not to specifically detect microbes, can provide an indication of the presence of microbiological contamination; these methods are reviewed in the following sections.

5.5.1.1 Visual assessment of samples

Prior to any analysis of samples, an initial visual assessment of the sample is essential, as this will provide guidance on the most appropriate selection of analytical method(s) to use during investigation of microbiological contamination. Visual examination is commonly known as the 'clear and bright' test (e.g. ASTM D4176 *Standard test method for free water and particulate contamination in distillate fuels-visual inspection procedures*), but standard procedures may be modified or optimised for observation of microbial material.

The sample should be allowed to settle. After settling, the appearance of the fuel layer, the water layer and the fuel:water interface should be noted. In particular, the sample should be examined for the presence of any indicators of microbiological contamination, such as:

– haze in the fuel layer;
– the presence of polymeric material at the fuel:water interface;
– the presence of a coherent 'mat' of material at the fuel:water interface;
– the presence of an emulsion at the fuel:water interface;
– free and/or turbid water, and
– soft particulates and/or sludge at the bottom of the sample.

Gentle swirling of the sample will assist in the visual examination by creating a vortex and disturbing any settled particulates/water drops into line of sight. Observation by eye of one or more of the attributes noted in this section should act as a trigger to investigate the possibility of microbial contamination.

It should also be noted that visual assessment in isolation should not be used as a reliable indicator for the presence of microbiological contamination. Observation by eye of microbial material will only be observed when large numbers are present; at which point significant fuel quality or spoilage is likely to have already occurred.

5.5.1.2 Water content

The presence of water is critical for controlling whether microbial growth will occur in fuel systems. To allow microbial growth, the water must be free water which is NOT dissolved in the fuel. However, most fuel contains some dissolved water, which may condense out as free water if the fuel cools.

As water is so critical to influencing the extent of microbial growth, determining the water content of fuel is recommended as part of routine monitoring or assessment of incidences of microbial contamination. Water content may be determined in the laboratory by Karl Fischer titration of samples, as described in standards IP 438 (coulometric method) or IP 439 *Petroleum products – Determination of water – Potentiometric Karl Fischer titration method*, both of which cover a detection range between 30 and 1 000 ppm water. Both methods allow for the addition of the surfactant sodium dioctylsulfosuccinate to fuel, so that the concentration of free and dissolved water in the fuel can be determined.

5.5.1.3 Other analytical methods

Several other analytical methods used to characterise petroleum products are useful indicators of the presence of microbial contamination; these include:

– pH. Due to the production and excretion of products of metabolism, microbial growth is often accompanied by a drop in aqueous phase pH. However, additives use to restore fuel properties lost due to refining and hydrotreating processes often partition into fuel-associated water and act as a buffer. Consequently, the observation of decreased pH is less useful than it was historically. However, as pH determination is a relatively easy and inexpensive test method, it is still recommended to test for pH whenever a significant water phase is observed in samples. Standardised methods such as ASTM D1293 Standard Test Methods for pH of Water, are available for determining pH of water.