

Fe(III) detection using thiocyanate

1. A stock solution of 100 mM NaSCN was prepared, along with a 500 mM stock of FeCl₃
2. To make the standard solutions, ATCC2039 media without Fe was taken, and FeCl₃ added to get the required concentrations. Used here were 100 mM, 30 mM, 10 mM, 3 mM and 1 mM.

[Fe ³⁺] (mM)	100	30	10	3	1
FeCl ₃ (500mM) volume (μL)	200	60	20	6	2
ATCC2039 volume (μL)	800	940	980	994	998

3. 100 μl of concentrated H₂SO₄ was added to acidify each sample.
4. The working reagent was made by diluting the NaSCN to 10 mM in water. 1 ml of working reagent was used per measurement. To this was added 20 μl of each acidified sample.
5. The absorbance at 450 and 546 nm was measured.
6. A standard curve was plotted.
7. At time points of 0, 12, 18, 24, 30 hr from a fresh 1/5 dilution of *Acidithiobacillus ferrooxidans* into new media, two 1-ml samples were taken.
8. 100 μl of conc. H₂SO₄ was mixed in, and the sample was clarified by filtration (0.2-μm pore).
9. 20 μl of each sample was added to 1 ml of working reagent
10. Absorbance at 546 nm was measured.