

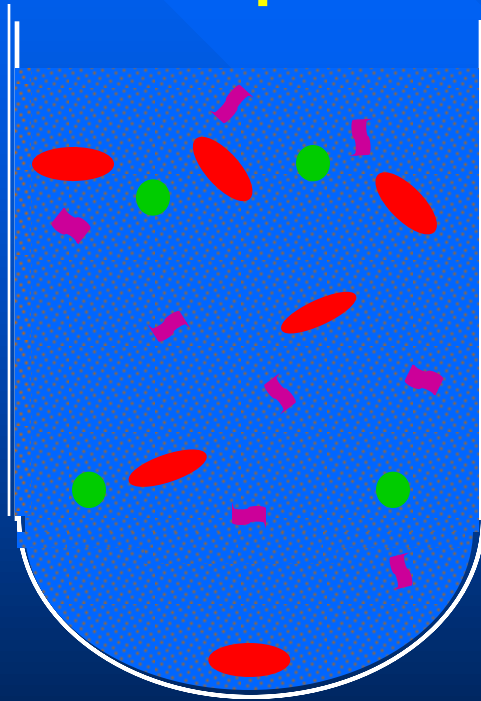
Development of an Immunomagnetic method based on IgG1 Monoclonal antibodies for *Cryptosporidium* and *Giardia*

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Mark Gauci, David Baer, James
Smith, Duncan Veal*

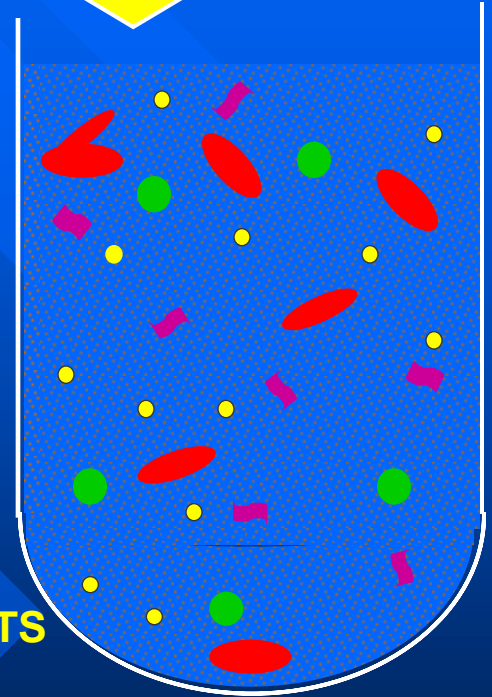
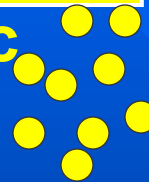


WATER CONCENTRATE

Concentrated Water
Sample



Add Magnetic
Beads



GIARDIA CYSTS



CRYPTOSPORIDIUM OOCYSTS



MAGNETIC BEADS

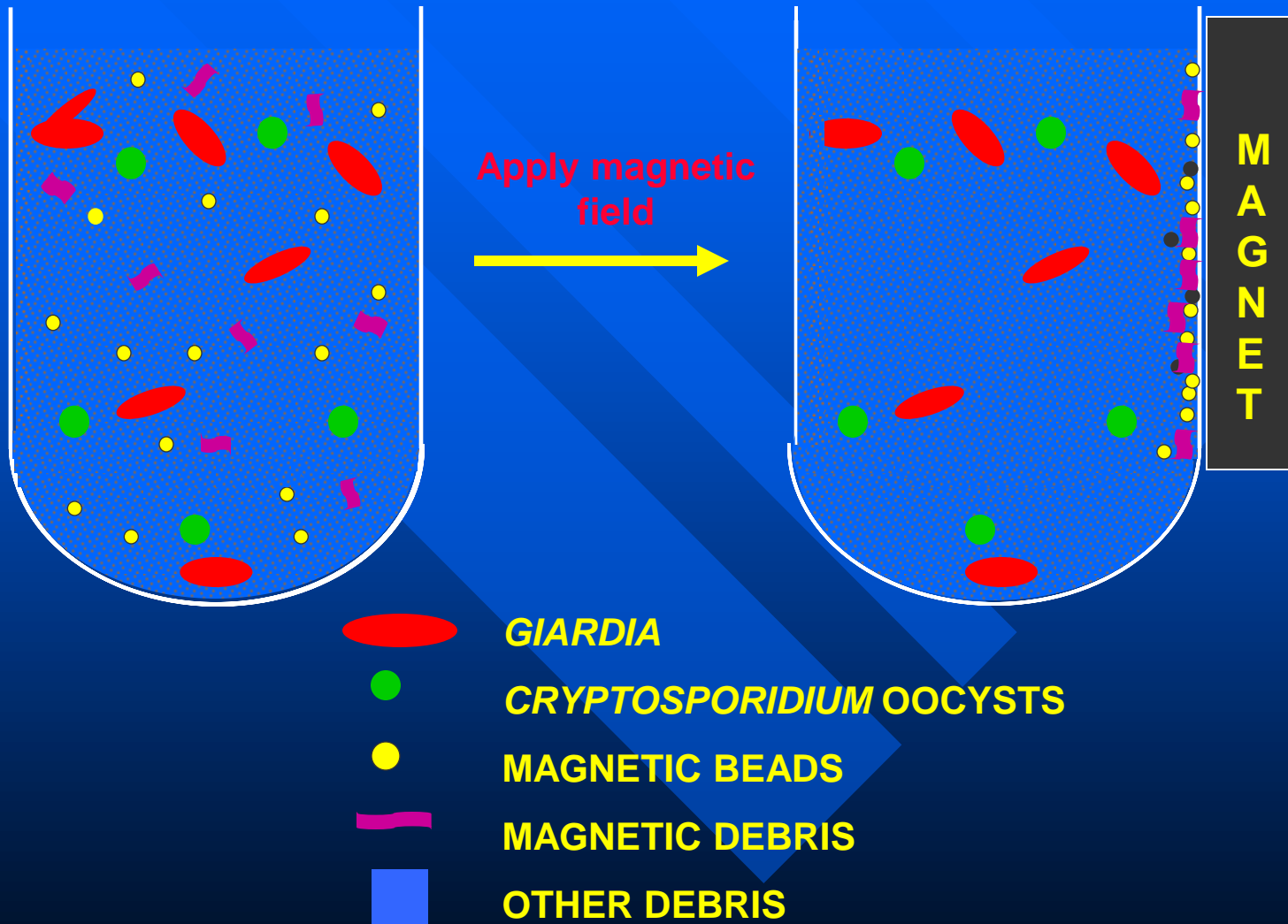


MAGNETIC DEBRIS

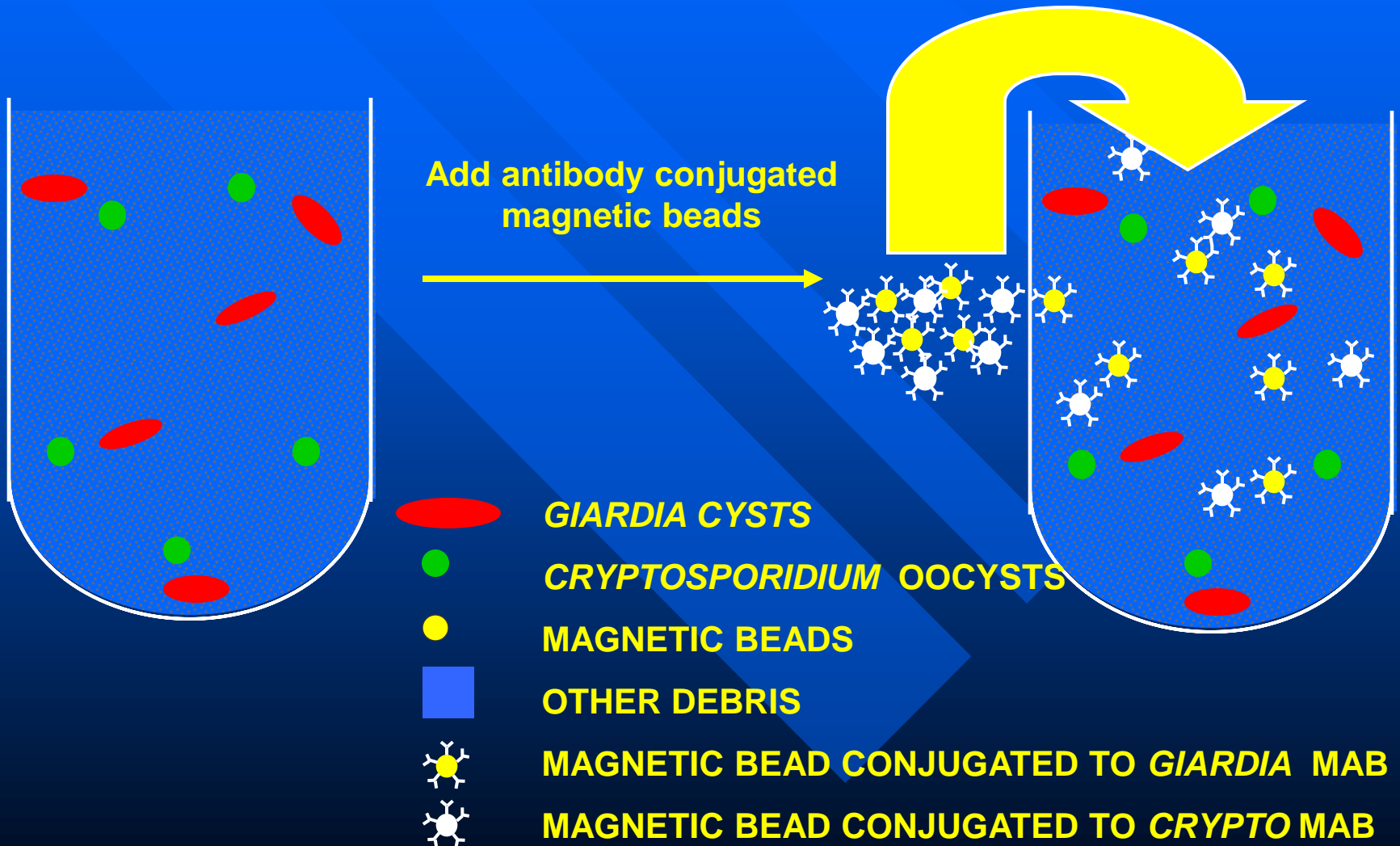


OTHER DEBRIS

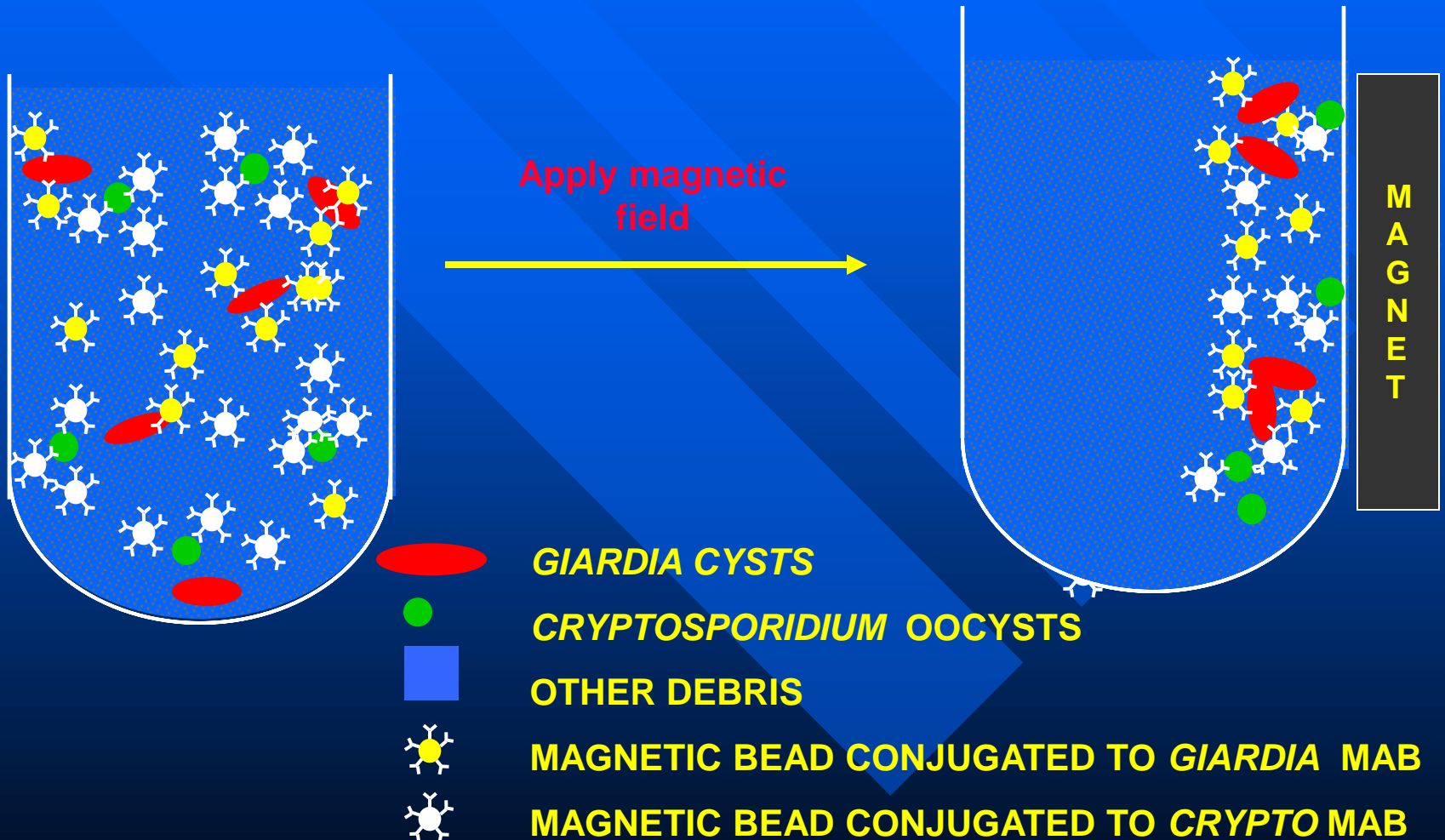
PRECLEAR



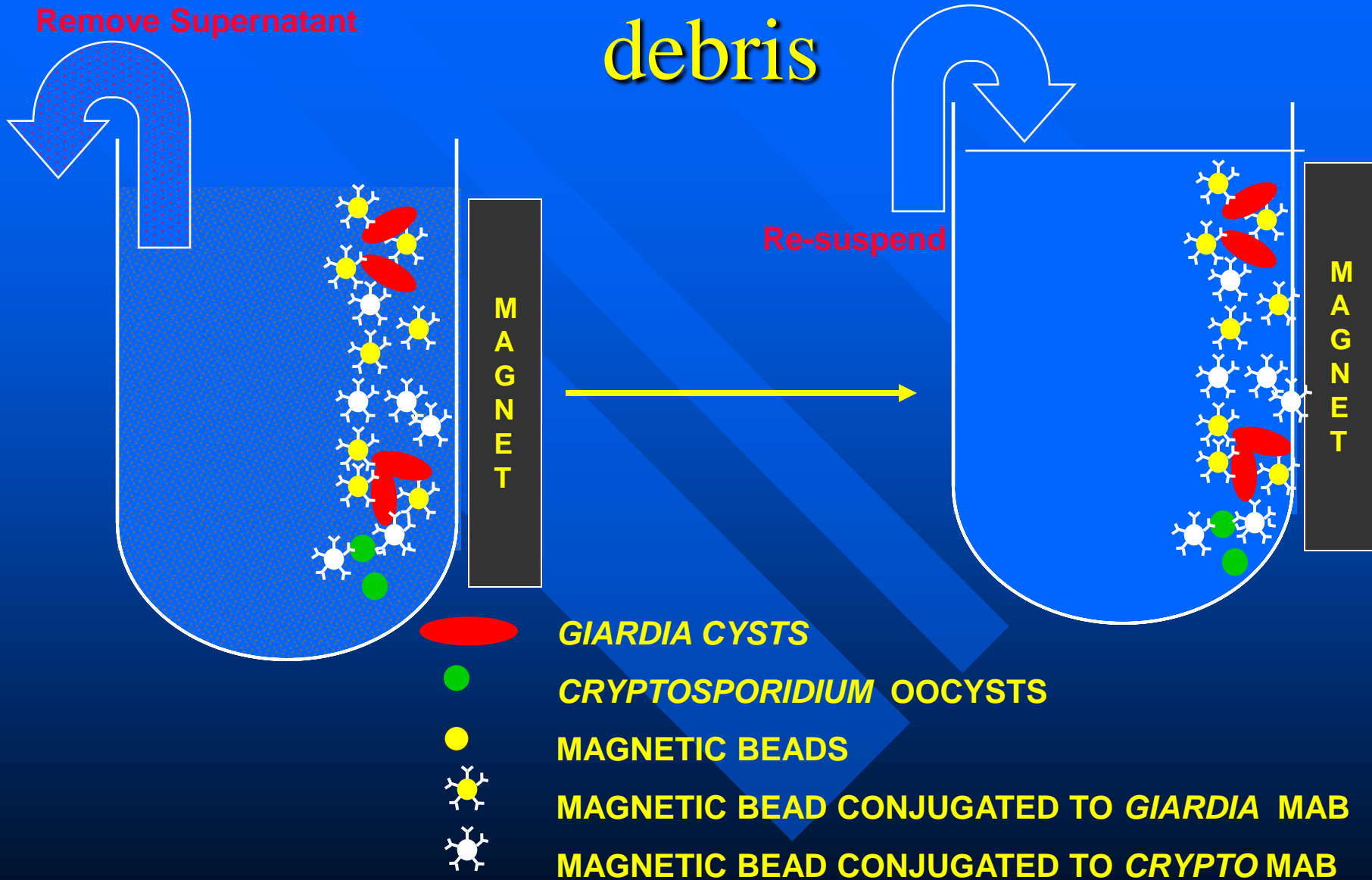
Addition of Conjugated Magnetic Beads



IMS OF *CRYPTOSPORIDIUM* & *GIARDIA*



Separation of (oo)cysts from debris



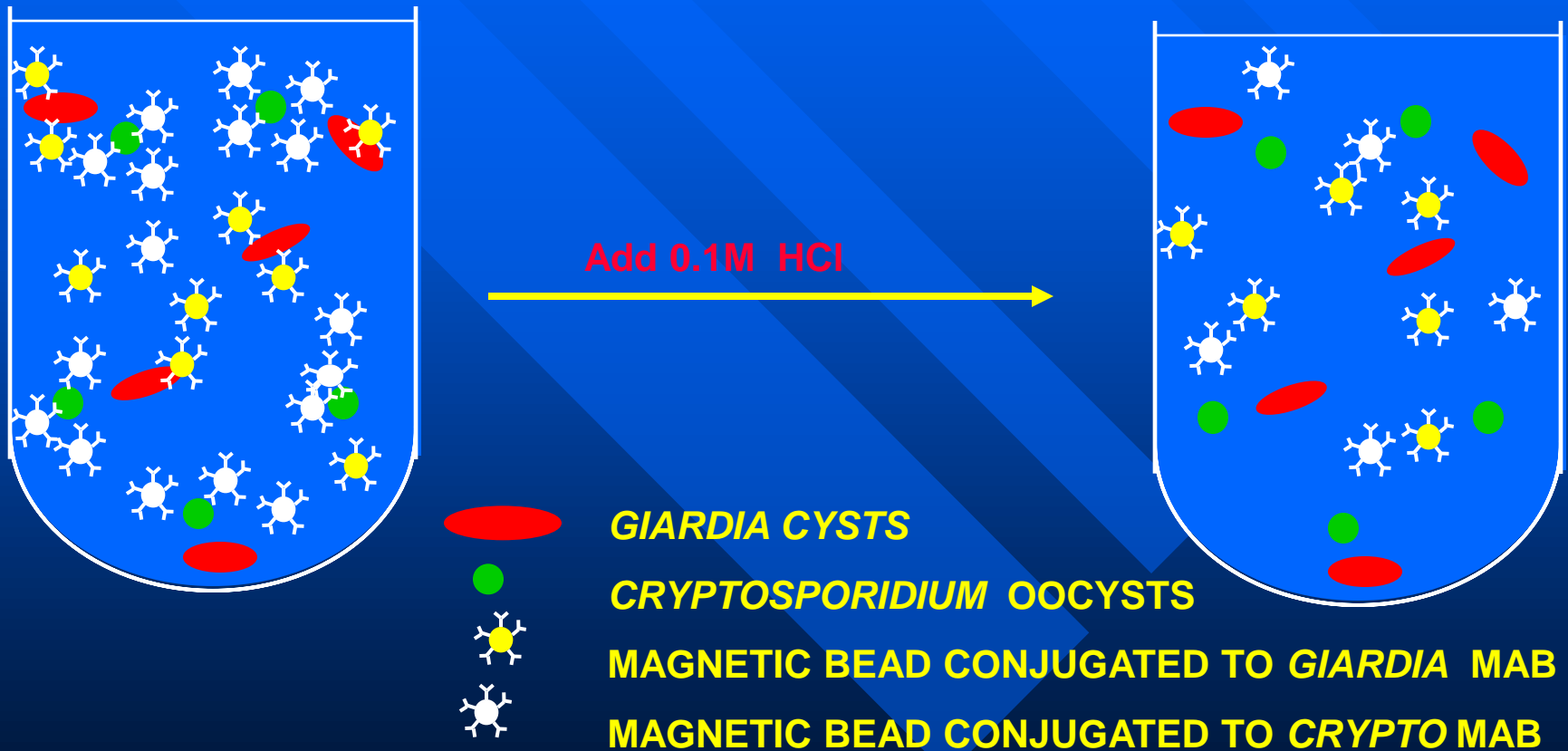


1

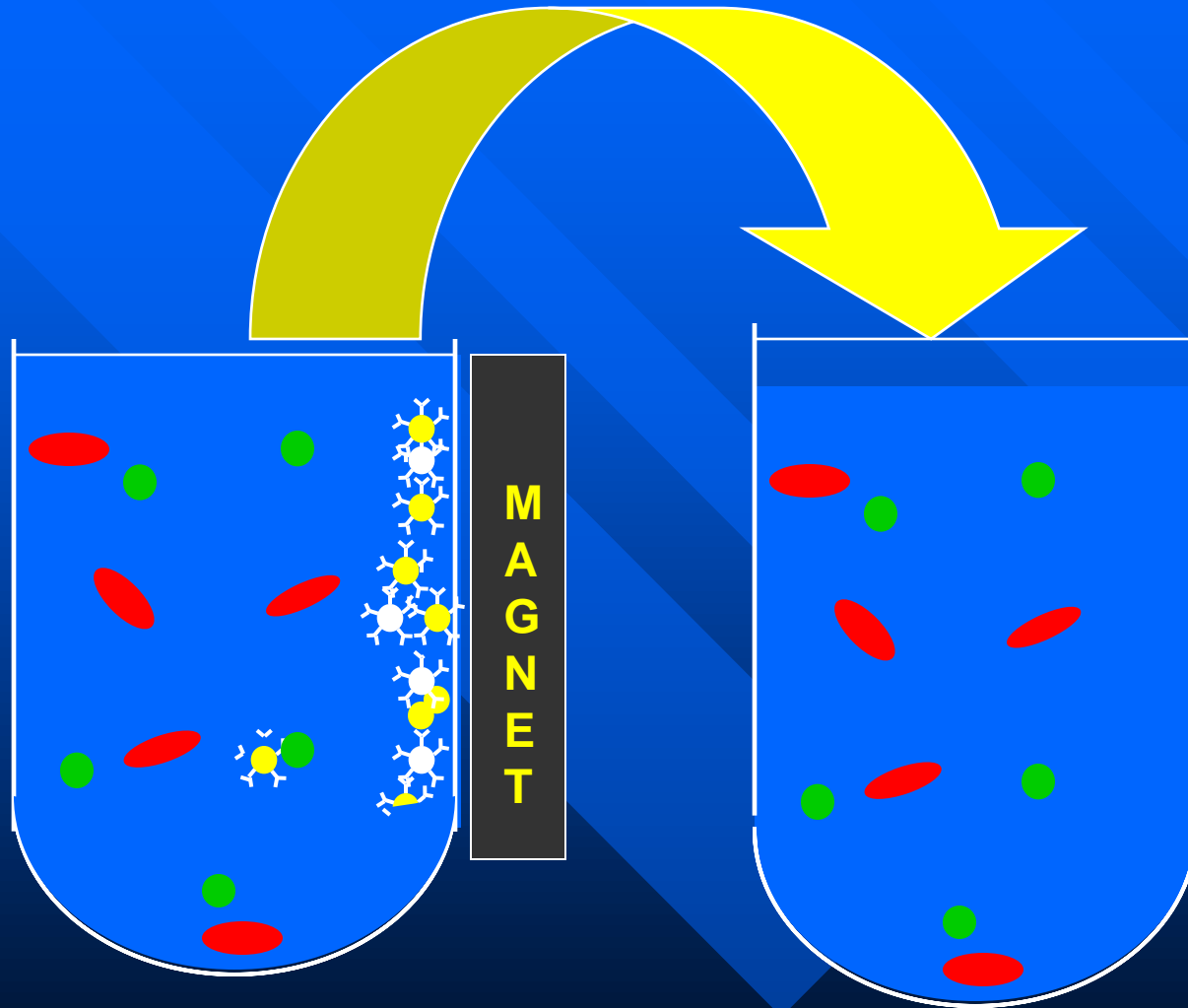
2

3

Acid Dissociation



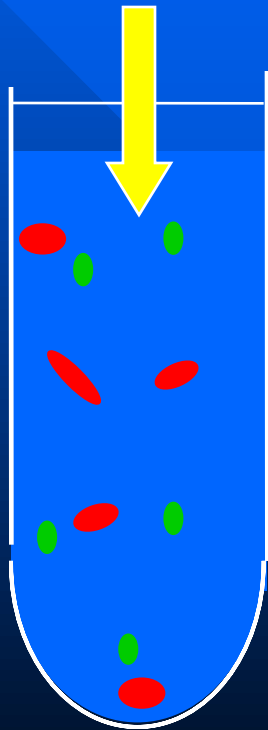
Acid Dissociation

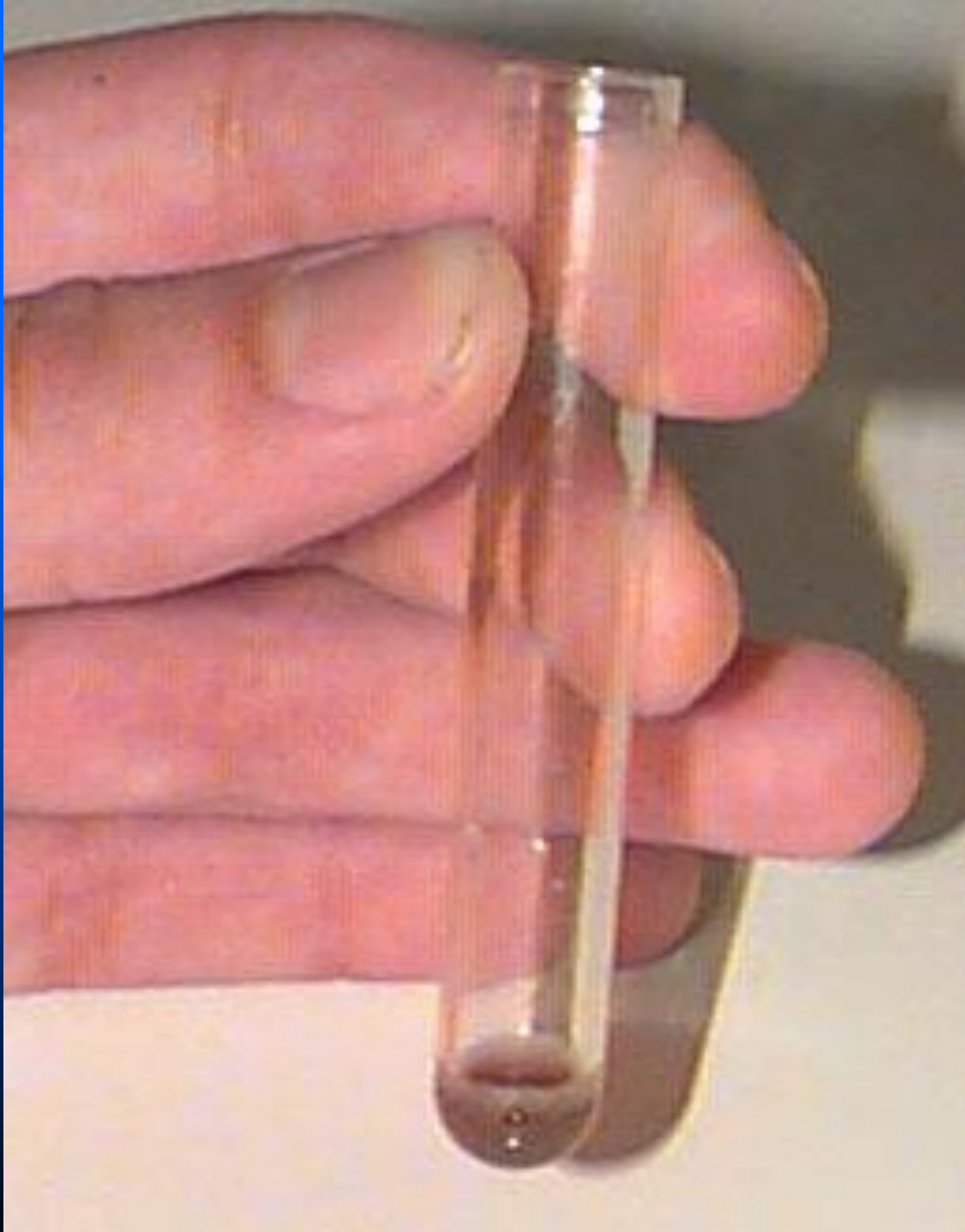


Identification

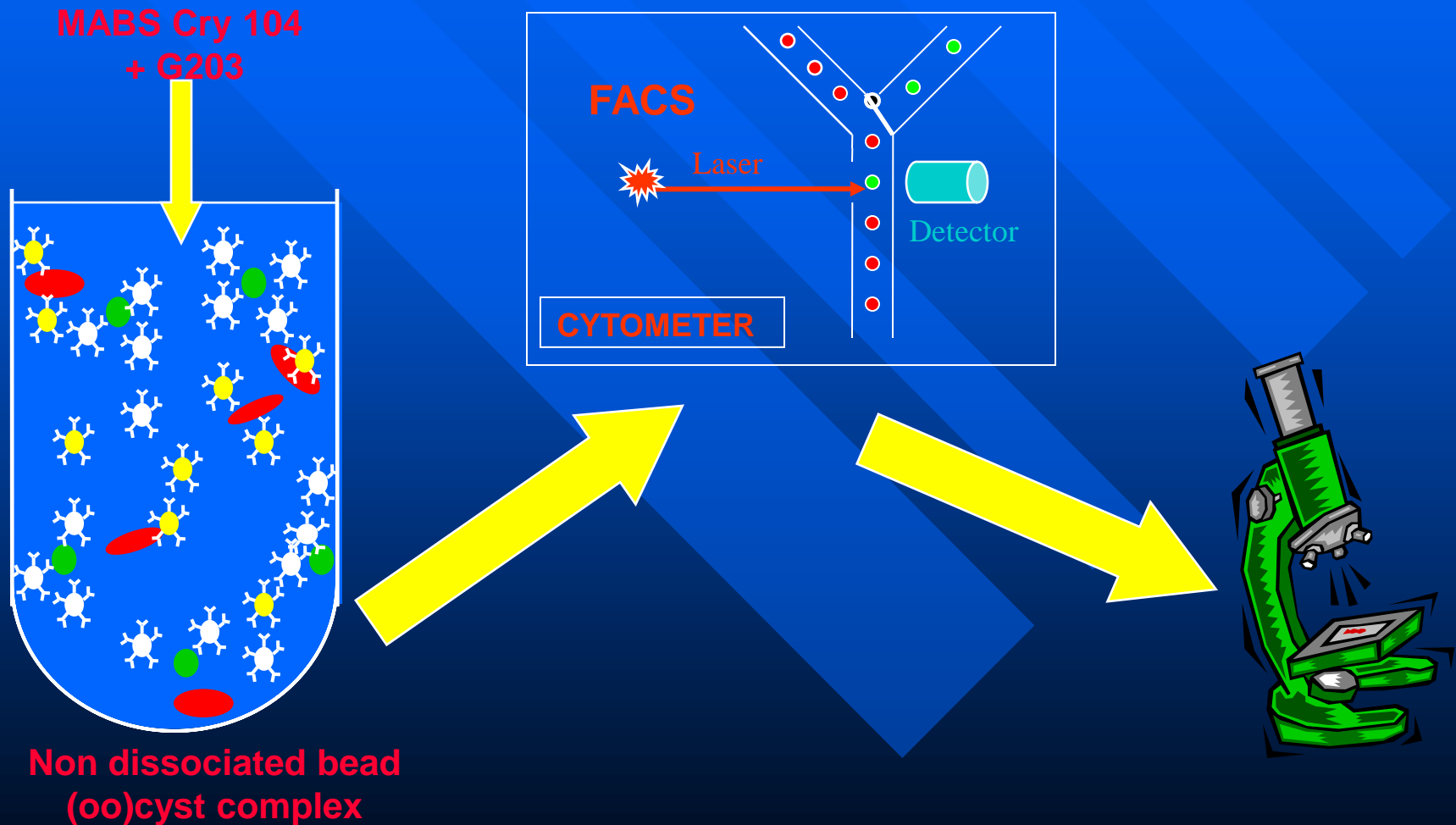
FLUORESCENT
MAB STAINING

MABS G203 +Cry 104





Analysis by flow cytometry



Recovery of oocysts from Wimallee Lagoon concentrated water sample and PBS using the Immucell™ IMS Kit.

	PBS	Wimallee Lagoon
Recovery (%)	36 ± 4.6	33 ± 6.4
DAPI Positive (%)	25 ± 4.0	21 ± 2.6
Time Taken microscopy (min)	17 ± 2.1	20 ± 1.5

Recovery of oocysts from Wimalee Lagoon concentrated water sample and PBS using the Dynal® IMS Kit

	PBS	Wimalee Lagoon
Recovery (%)	47 ± 6.5	36 ± 3.4
DAPI Positive (%)	28 ± 4.9	25 ± 3.3
Time Taken microscopy (min)	19 ± 2.6	20 ± 2.0

Beads and antibodies

Beads	Size (μm)	Supplier	Cost beads / ml	<i>Cryptosporidium</i> and <i>Giardia</i>-specific mAb
Dynal® IgG	4.5	Dynal®,	\$145	CRY 104 (Mac Uni) OW50 (Uni Arizona); <i>Giardia</i> -specific mAb G203 (Mac Uni)
Biomag	1	Perspective Diagnostics	\$6	CRY 104 (Macquarie University)
Spherotech	4-4.5	Spherotech,	\$20	CRY 104 (MacUni)
Dynal® IgM	4.5	Dynal®,	\$145	CRY 26 (Mac Uni)

Recovery of oocysts from Wimalee Lagoon using 0W50-coated anti-IgG Dynal® beads and CRY 26-coated anti-IgM Dynal® beads.

	0W50-coated anti-IgG Dynal® beads	CRY 26-coated anti-IgM Dynal® beads.
Recovery (%)	71 (\pm 12.8)	56 (\pm 17.5)
Time Taken FACS (min)	5.2 (\pm 0.4)	5.9 (\pm 0.6)
Time Taken microscopy (min)	8.8 (\pm 1.2)	7.7 (\pm 0.4)

**Comparison of recovery of oocysts from Wimallee Lagoon
performing IMS with CRY 104-coated Spherotech™,
Biomag® and Dynal®-anti-IgG beads.**

	Biomag®	Dynal®	Spherotech™
Recovery (%)	91 (\pm 3.1)	94 (\pm 3.4)	79 (\pm 7.5)
Time Taken FACS (min)	11.4 (\pm 2.3)	10.1 (\pm 1.4)	12.7 (\pm 1.2)
Time Taken microscopy (min)	12 (\pm 1.0)	13 (\pm 1.5)	12.3 (\pm 1.5)

Recovery of oocysts from different water types using CRY 104-coated Dynal® beads.

Water Sample	Original sample volume (L)	Recovery (%)	Time Taken FACS (min)	Time Taken microscopy (min)
Wimalee Lagoon	10	90 (± 3)	10.3 (± 2.2)	14.3 (± 2.5)
Drinking Water	100	78.3 (± 5.5)	4.5 (± 1.0)	19.3 (± 5)
River Water	100	92.7 (± 5.8)	5.9 (± 2.1)	14.3 (± 2.5)

Recovery of *Giardia* cysts from different water types using G203-coated Dynal® anti-IgG beads

Water Type	Recovery (%)	Time Taken FACS (min)	Time Taken microscopy (min)
Wimalee Lagoon	86.3 (\pm 5.5)	5.1 (\pm 0.5)	3.8 (\pm 0.2)
Drinking Water	87 (\pm 7.9)	5.5 (\pm 0.5)	5.4 (\pm 0.2)
River Water	78.3 (\pm 11.9)	5.6 (\pm 0.7)	4.4 (\pm 0.5)