Magnetic Quantum Dots for Cell Separation Using the CoreMag2T Magnet

Background

Funded by research through the National Science Foundation (NSF), the MagDot is a unique nanoparticle that combines both quantum dots and superparamagnetic iron oxide particles within the same nanoparticle. The MagDot is therefore both fluorescent and magnetic. This duality allows one using the MagDots to perform magnetic cell separation on MagDot-labeled cells and then transfer the magnetically enriched or depleted cells immediately to a fluorescence-based application, such as flow cytometry.

In addition to MagDot manufacturing for the NSF, we proposed the creation of an open magnetic separation system to collect MagDot-labeled samples in a timely manner. An "open" magnetic separation refers to the absence of packed beads in a magnetic separation column, which contrasts with most commercial magnetic separation systems. Packed bead configurations are used to increase the magnetic fields applied to immunomagnetically labeled cells. In the presence of a magnetic field, these metal beads become magnetized, and because of their spherical geometry, high magnetic gradients are induced in the interpolar gaps. This configuration is favored because it is inexpensive and overcomes the magnetization limitations of small commercial magnetic nanoparticles. These architectures induce clogging, which is a much greater problem in tissue cell suspensions and other applications where samples are highly heterogeneous and viscous. We therefore developed an open magnetic separation system with Dexter Magnetic Technologies for use with the MagDot reagents.

Magnetic cell separation

In the last two decades, magnetic cell separation, facilitated by using paramagnetic colloids conjugated to antibodies, has become widely used for the separation and isolation of common and rare cell populations. Three main parameters have an impact on an effective magnetic cell isolation: 1) the degree to which the cell is labeled with the magnetic particles, 2) the magnetic content of the label, and 3) the field strength of the magnet. The measure of the degree of immunomagnetic labeling is a quantity called the cell's magnetophoretic mobility. The magnitude of a cell's magnetophoretic mobility has been shown to affect the performance of a commercial column magnetic cell separation system as well as an open magnetic system. The magnetic separation speed of a cell labeled with magnetic nanoparticles in a magnetic field *V,* can be expressed by equation 1 [1].

$$V = \frac{\Delta\chi V_m \beta ABC}{3\pi D_c \eta} S_m \qquad \text{Eq. 1}$$

where, $\Delta\chi$ is the difference in magnetic susceptibility between the carrier and the suspending medium;
V_m is the volume of the paramagnetic material;
βABC represents the magnetic particle binding capacity of the cell (the antibody binding capacity);
D_c is the diameter of the cell;
η is the viscosity of the fluid;
and S_m is the magnitude of the magnetic gradient
Where: $S_m = \frac{|\nabla B^2|}{2\mu_0} = |\nabla E|$; $E = \frac{B^2}{2\mu_0}$; *Therefore*, $V \propto |\nabla E|$.
The magnetophoretic mobility is therefore proportional to the magnetic content of the particle and to the gradient of magnetic energy of the magnet used for separation. This gradient is also the force density of the magnet.

MAGDOTS

Magdot content and its formulation parameters were defined after interviews with potential customers that revealed the following list of features most desired in cell sorting and analysis techniques employed: 1) high throughput, 2) ability to process large volumes of samples, 3) high purity, 4) high cell recovery, 5) maintenance of cell viability, i.e. gentle separation, 6) ease of implementation and 7) cost. To meet these design criteria, we optimized the MagDot formulation, particularly size, magnetic loading, and quantum dot emission wavelength to match the demands of the cell separation industry.

Formulation of MagDots

MagDots are formulated using a liquid-in-liquid electrospray process (LLE) as shown in Figure 1a. A nonconductive, water-miscible, organic liquid (i.e., THF), containing polymers, superparamagnetic iron oxide nanoparticles (SPIONS) and quantum dots (QDs) undergo turbulent mixing in distilled water in the presence of electrical stresses. Electric field established between the needle and ground wire causes the motion of the charge carriers within the continuous aqueous phase, resulting in kinetically driven nucleation and growth of colloidally stable polymer nanocomposites. In LLE driven nanoprecipitation process, the QDs, SPIONs and block polymer in THF are sprayed into water to obtain MagDots with uniform and narrow size distribution. The hydrophobic portion of the polymer (polystyrene) encapsulates and binds the SPIONs and the QDs together. The hydrophilic part of the polymer (polyethylene glycol, PEG) allows for water solubility contributing to colloidal stability and the ability to be conjugated to antibodies and other proteins of interest through standard conjugation chemistries by use of the terminal functional groups on the PEG chains.

Characteristics of the MagDots

Transmission electron microscopy of the MagDots confirms the structure of the MagDots and the encapsulation of both components is seen in Figure 1b. The narrow size distribution of the particles allows for uniform labeling and detection of the receptors on cells. Figure 1c shows the different emission spectra possible with the MagDot reagents. The currently available MagDot emissions are 580 nm, 610 nm, 640 nm and 655 nm. There is the possibility to add colors at wavelengths every 20 nm. The emission spectra are extremely narrow with full width of half maximum averaging 24 nm.

Antibodies conjugated to MagDots are reduced using mercaptoethanol to obtain thiol groups. The thiol group is then reacted with Sulfo TCO-Maleimide and reacted with Tetrazine-activated

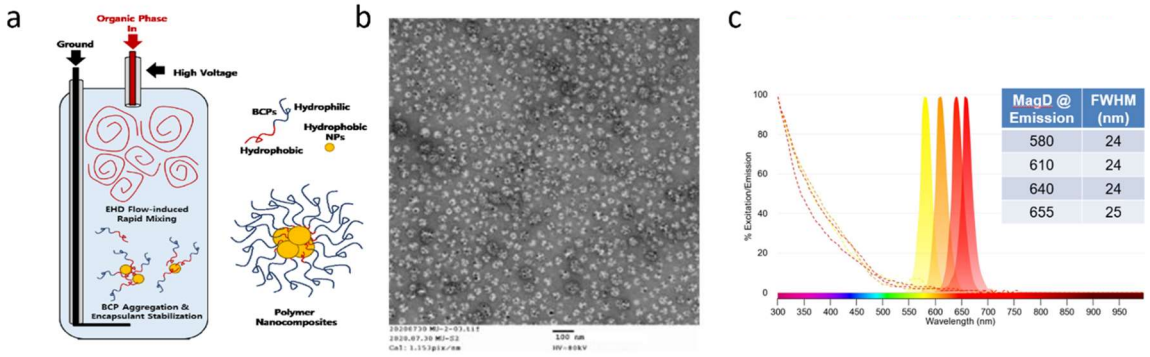

Figure 1. a. LLE setup for formulation of Magdots b. TEM images of the MagDots, c. Emission spectra of MagDots excited at a 405 nm laser

MagDots to obtain antibody conjugates. Unbound antibodies are purified using magnetic collection and washing. Post antibody conjugation, the hydrodynamic size of the MagDots are 120 nm with PDI < 0.2 as measured in a Brookhaven Nanobrook Dynamic Light Scattering system. There is a 10-20 nm increase in the hydrodynamic size of the initial MagDot size with the conjugation of the antibodies.

Iron Concentration of MagDots vs other commercially available magnetic nanoparticles

Figure 2 shows how the MagDot iron concentration compares to commercially available particles. Iron concentrations normalized by mass of our formulations are comparable (BD Bioscience magnetic only nanoparticles) or much higher to the commercially available magnetic nanoparticles (Stem cell and Miltenyi magnetic only nanoparticles).

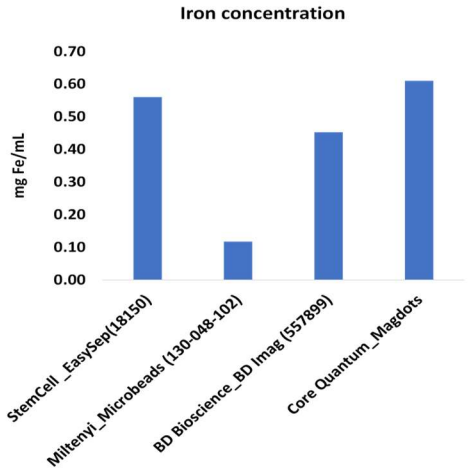

Figure 2. Comparison of the particles from Core Quantum to commercially available magnetic only nanoparticles.

MAGNET DESIGN

The separation speed of the MagDots formulated were tested in a quadrapole design magnet. Cell Tracking Velocimetry (CTV) measurements which measure the velocity of magnetically labeled cell in a defined magnetic field gradient indicated that cells labeled with MagDots had a velocity of 6.8 microns per second in Model 1014 magnet designed by the Cleveland Clinic Department of Biomedical Engineering.

With a velocity of 6.8 um/s of MagDot labeled cells, it would take ~ 30 minutes for separation in magnet with a 1.4 T field strength (Figure 3). Typical times for magnetic separation that are acceptable are between 15 and 20 minutes.

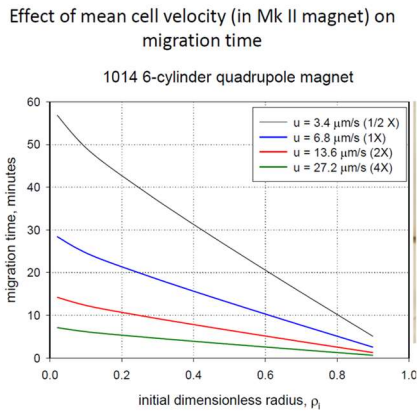

Figure 3. Theoretical separation times in magnets designed with model 1014.

Rather than increase the amount of SPIONS loaded in the MagDots, V_m from equation 1, jeopardizing colloidal stability and increasing overall size, we opted to manufacture a magnet with a higher magnetic gradient, S_m, thereby increasing the gradient of magnetic energy.

General Quadrupole Magnet

A general quadrupole design derived from the Halbach array [2] is illustrated in Figure 4a below. For a quadrupole magnet with OD of 60 mm, ID of 13 mm and length of 60 mm, the magnetic field and energy at the center plane are shown in Figure 4b. Peak magnetic field reaches around 1.846 Tesla at the pole faces. Figure 4c shows the energy density and force density plots from one pole to the center of the cavity.

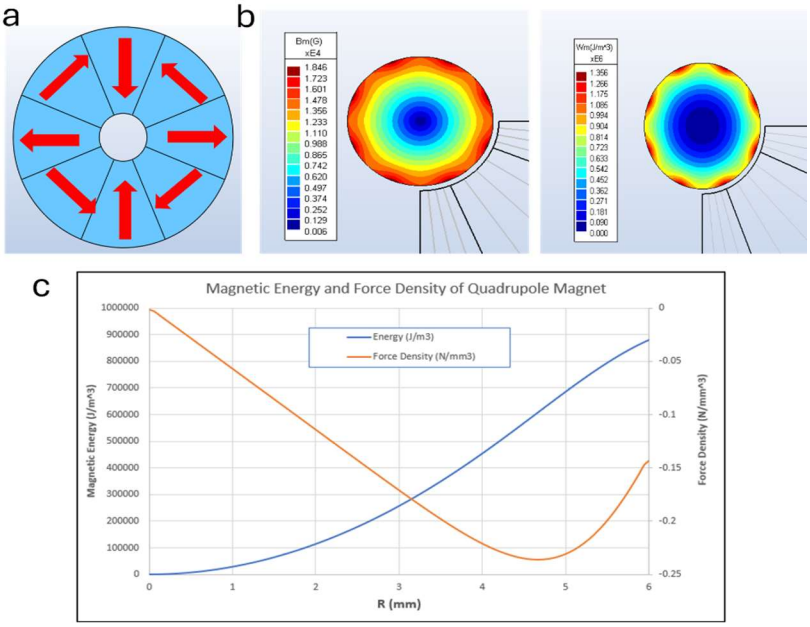

Figure 4. a. Quadrupole magnet with OD of 60 mm, ID of 13 mm and length of 60 mm. b.Magnetic field and energy plot on center plane (plotted on OD=12 mm circle) c. Energy and force density plots.

Model 1014

The NSF project resulted in a collaboration with the Cleveland Clinic Foundation Biomedical Engineering department. Dr. Maciej Zborowski designed the original quadrupole magnet discussed previously and thought that a Halbach array style deemed the Model 1014 (Figure 5) would be a useful design for the MagDots. Unfortunately, due to the pandemic, design and production was slow. Dr. Zborowski ultimately retired from the Cleveland Clinic and therefore we looked for a new collaborative partnership with Dexter Magnetic Technologies to help design and manufacture a magnet that would meet our specifications.

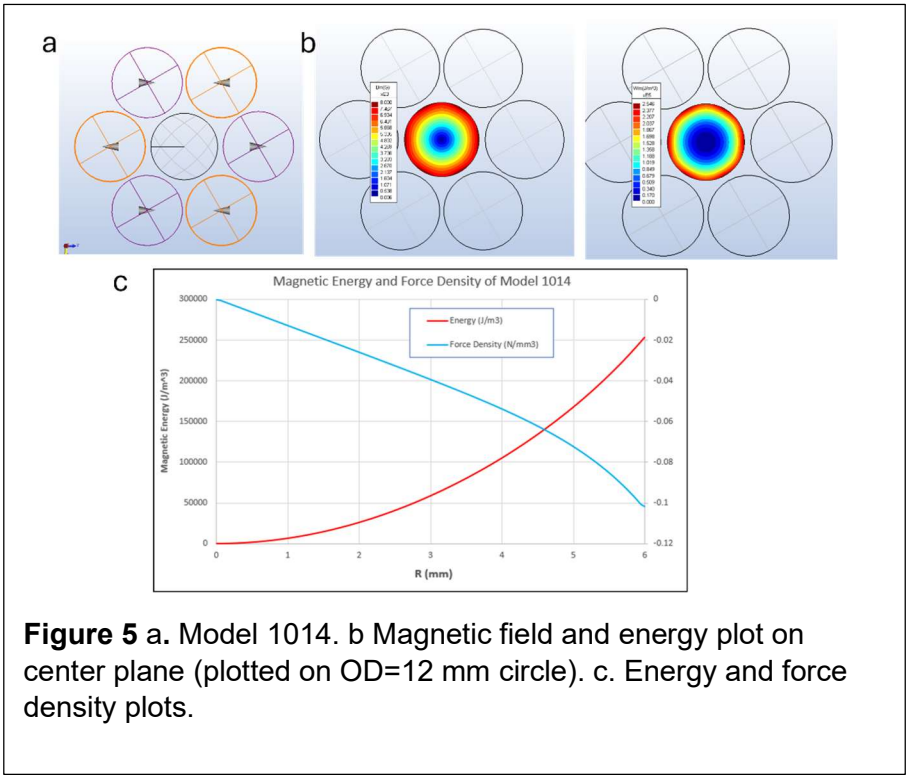

Figure 5 a**.** Model 1014. b Magnetic field and energy plot on center plane (plotted on OD=12 mm circle). c. Energy and force density plots.

Need for Dexter's Magnet Design

The request to manufacture a magnet design from Dexter was to obtain a field strength of nearly 2 Tesla to be able to collect MagDot labeled cells within 15 to 20 minutes. In addition to a high field strength, the request to have a semi-circle design was proposed. A semi-circle design, or open access, would allow the user to see where the pipet tip is going to not disturb magnetically collected cells. The design was modeled against other magnetic designs such as the general quadrupole magnet that has been used for magnetic separations in the past.

Dexter's Magnet Design with Open Access

To allow visual access for the user, one pole is removed from the quadrupole magnet and the two side poles are modified. A steel housing is added to reduce stray field. The open access magnet is illustrated in Figure 6a. Figure 6b shows the magnetic field and energy density plots. Peak field reaches around 2.06 Tesla at the pole face. The active field inside the vial decreases to around 1.5 T due to the air gap. Figure 6c shows the energy density and force density from the main pole to the center of the cavity.

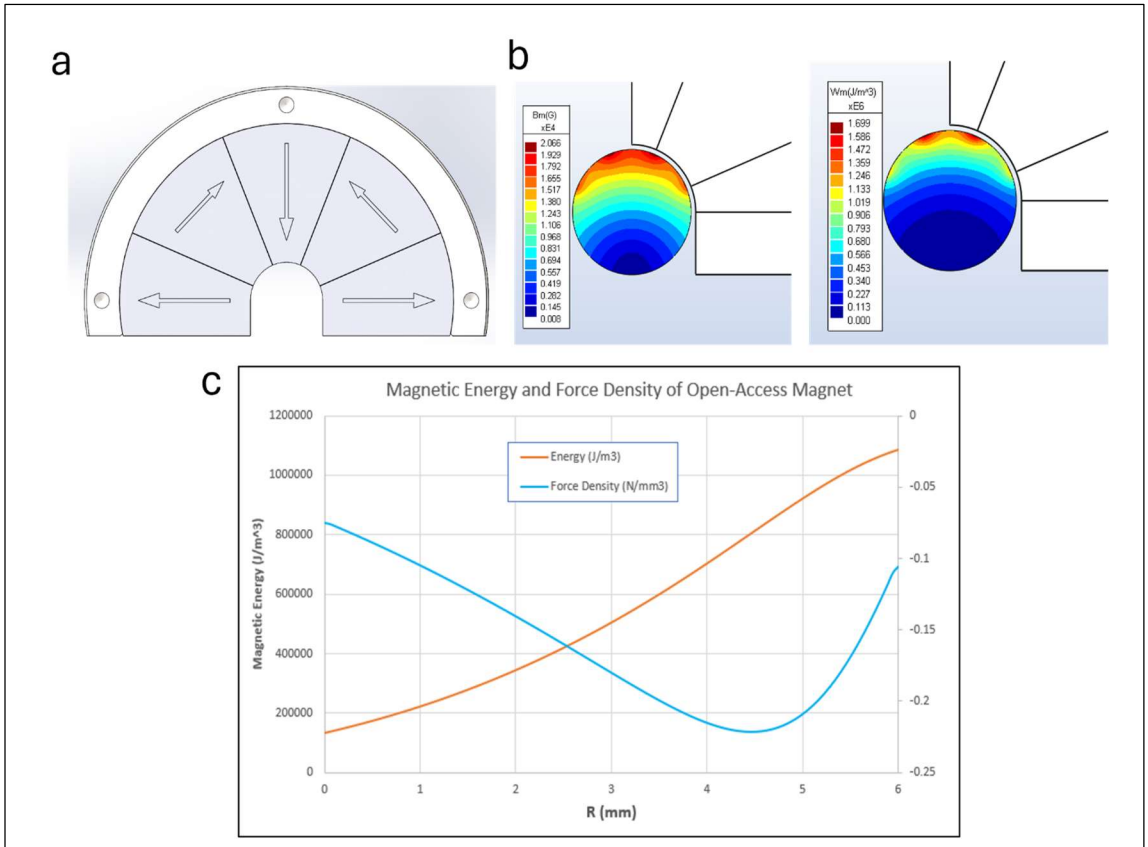

Figure 6 a.Magnet design with open access. b. Magnetic field and energy plot on center plane (plotted on OD=12 mm circle). c. Energy and force density plots.

The force density of the open access magnet is about 4 times of the model 1014 within the usable range and slightly higher than the traditional quadrupole magnetic design.

The force density of a general quadropole design to the open access magnet designed by Dexter is tabulated below. The force density confirms that the magnetophoretic mobility of the MagDots in an open-access magnet would remain similar to a fully circular conventional magnet.

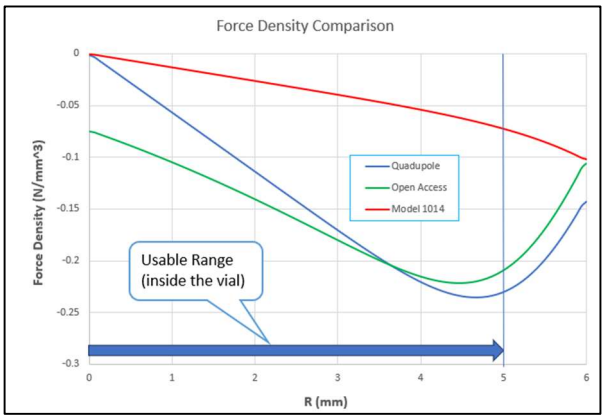

Figure 7. Force densities compared

Design	Maximum Magnetic Field (T) at R=6 mm	Maximum Force Density (N/mm^3)
Quadrupole	1.846	0.236
Dexter -Open Access	2.066	0.222
Model 1014	0.8	0.102

Table 1: Comparison of the force densities of multiple designs

The resulting commercially available CoreMag2T as designed is shown in Figure 8.

Figure 8. The Core Quantum Technologies, CoreMag2T Magnet.

Cell Separation Using MagDot labeled cells in CoreMag2T Magnet

MagDots with anti-human CD4 (clone RPT4) have been developed for the separation of human cells based on the expression of the CD4 antigen. Below is an example how CoreMag2T was used to enable rapid and effective separation of MagDot-labeled cell suspensions within minutes.

Materials

1. CytoComp cells
2. Mouse anti-human CD4 biotinylated antibody
3. Streptavidin conjugated 610nm MagDots
4. Buffer: PBS + 2mM EDTA + 0.5% BSA
5. Pipetman
6. Pipet tips
7. CoreMag2T Magnet
8. 5 ml flow cytometry tubes

Methods

1. Cytocomp cells were resuspended in their provided serum, to which the PBS buffer was added and cells were washed by centrifugation for 7 minutes.

2. The supernatant was discarded, and the pellet was resuspended in 1 mls of PBS buffer so that the cell concentration was 1750 cells/ul.

3. One million cells were removed and 10 ul of biotinylated mouse anti-human CD4 antibody was added. Cells and antibody were incubated for 30 minutes at room temperature.

4. After incubation period had expired, cells were washed by centrifugation for 7 minutes.

5. Supernatant was discarded and cells were resuspended in 200 ul of the PBS buffer.

6. To the resuspended cells, 100 ul of streptavidin conjugated 610nm MagDots were added. MagDots and cells were incubated at room temperature for 30 minutes.

7. Once incubation period had expired, the volume of the cell suspension was increased to 500 ul by adding additional buffer. An aliquot of 100 ul was removed from the increased volume, and placed in a separate tube called the feed (Figure 1). The feed is the fluorescent measurement of the cell suspension labeled with MagDots prior to the magnetic separation.

8. The remaining cells in 400 ul were placed into the bore of the CoreMag2T magnet for 20 minutes.

9. Once 20 minutes had passed, the unbound cells were aspirated from the tube while still in the magnetic field and placed in a tube labeled CD4 negative cells.

10. The tube was then removed from the magnet to which 400 ul of the PBS buffer was added and then the tube was vortexed. All samples were then run on a Becton Dickinson Fortessa flow cytometer using the BV605 filter off the 405 nm (violet) laser.

Results

In a one step labeling process, 97% of cells were recovered in the magnetic fraction with a purity of almost 90%. Figure 14a shows the fluorescent population of CD4 positive cells before the magnetic separation. Figure 14b shows the magnetic fraction containing the fluorescently/magnetically labeled cells with a purity of 86%, and Figure 14c shows the nonmagnetic fraction with 90% of the cells negative for CD4.

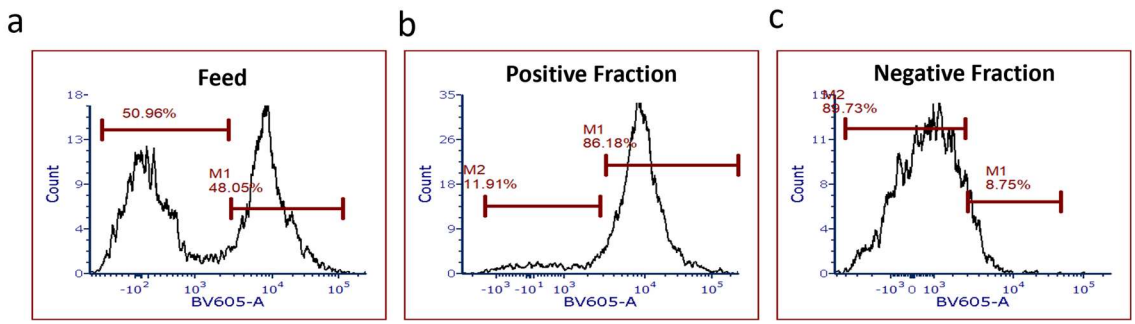

Figure 14. Cells labeled with anti-CD4 610 MagDots prior to magnetic separation. b. Magnetic fraction – cells positive for CD4 receptor. c. Nonmagnetic fraction – cells negative for CD4 receptor.

References:

[1] K. Melnik, M. Nakamura, K. Comella, L. C. Lasky, M. Zborowski, and J. J. Chalmers; Evaluation of Eluents from Separations of CD34+ Cells from Human Cord Blood Using a Commerical, Immunomagnetic Cell Separation System; Biotechnol. Prog. 2001, 17, 907−916

[2] K.Halbach, “Design of permanent multipole magnets with oriented rare earth cobalt materials”, Nucl. Instru. and Methods, Vol.169, pp 1-10 (1980)